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AAR1/TUP1 Protein, with a Structure Similar to That of the β Subunit of G Proteins, Is Required for a1-α2 and α2 Repression in Cell Type Control of *Saccharomyces cerevisiae*

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We have cloned a DNA fragment complementing the *aar1* mutation defective in the a1-α2 repression of the *α1* cistron and haploid-specific genes in *Saccharomyces cerevisiae*. Nucleotide sequence and mapping data indicated that the *AAR1* gene is identical with *TUP1*, which is allelic to the *SFL2*, *FLK1*, *CYC9*, *UMR7*, *AMM1*, and *AER2* genes, whose mutations are known to confer a variety of phenotypes, such as thymidine uptake, flocculation, insensitivity to glucose repression, a defect in UV-induced mutagenesis, and a defect in *ARS* plasmid maintenance. The TUP1/AER2 protein is known to have significant similarity with the β subunits of G proteins in the C-terminal half, in two glutamine-rich domains in the N-terminal half, and in a central region rich in serine and threonine residues. Disruption of the chromosomal *AAR1* gene in α and a/α cells conferred the nonmating phenotype, and the a/α diploids could not sporulate. The *AAR1/TUP1* gene is transcribed into a 2.5-kb mRNA independently of the mating-type information of the cell. These observations and mRNA analysis of cell-type-specific genes indicated that the AAR1/TUP1 protein is also indispensable for a1-α2 repression of *RME1* and for α2 repression of a-specific genes.

Haploid cells of *Saccharomyces cerevisiae* differentiate into two mating types, **a** and α. The **a** and α haploid cells mate with each other and produce **a**/α diploid cells of a third cell type, which does not mate but can undergo meiosis and sporulation. These cell types are determined by two alleles or idiomorphs (22), **a** and α, in the mating-type locus *MAT* on the right arm of chromosome III (14). Cells with the *MAT***a** allele exhibit the **a** mating type, those with the *MAT*α allele exhibit the α mating type, and those with both alleles exhibit the third cell type. In addition to the *MAT* locus, there are two other genes on the same chromosome, *HML* and *HMR*, that provide mating-type information. The *HML* and *HMR* loci generally contain α and **a** information, respectively, but their expressions are repressed by the *SIR* genes. The *MAT*α gene encodes two DNA-binding proteins, α1 and α2 (1, 16). α1 activates the α-specific genes (α1 activation), and α2 represses the **a**-specific genes (α2 repression). The *MAT***a** gene encodes two proteins, **a**1 and **a**2, which have no function in haploid cells; however, **a**1 bears a homeo domain (37) and, in combination with α2, represses the transcription of the α1 cistron, the haploid-specific genes, Ty1 elements (8), and the *RME1* gene (23) (**a**1-α2 repression). The *RME1* gene product is a negative regulator of the sporulation-specific genes. No particular functions of the **a**2 protein have been detected.

In a previous communication, we described two classes of mutants showing defective **a**1-α2 repression but normal α2 repression (13). One of the classes had mutations in the α2 protein, and the mutant alleles were designated as *hml*α*2-102*, *mat*α*2-201*, and *mat*α*2-202*. The other class had mutations designated as *aar1* (**a**1-alpha2 repression).

Here, we report the cloning and molecular analysis of the *AAR1* gene. Comparison of nucleotide sequences indicated that *AAR1* is identical with the *TUP1* (51), *SFL2* (9), and *AER2* (54) genes. Disruption of the *AAR1/TUP1* gene conferred the nonmating type on α and **a**/α cells and prevented sporulation of **a**/α diploid cells. These facts strongly suggest that the AAR1/TUP1 protein, with a structure similar to that of the β subunit of G proteins (51, 54), is a component of regulatory complexes for **a**1-α2 and α2 repression in cell type control.

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MATERIALS AND METHODS

Microorganisms and plasmids. The *S. cerevisiae* strains used are listed in Table 1. All the strains were constructed in our laboratory and had the *ho* genotype. Two *Escherichia coli* strains, JA221 (3) and MV1184 (48), were used as hosts for propagation and manipulation of plasmids. The plasmid vectors used were YCp50, YIp5, YRp7, and YEp13 (27) and pUC118 and pUC119 (48). Plasmid pHK104, constructed in a previous study (18), was used as a source of the *MF*α*1-PHO5* fusion gene. A library of *S. cerevisiae* genes, YCp50 CEN BANK A, constructed by partial digestion of genomic DNA of *S. cerevisiae* with *Sau*3AI and its ligation into YCp50 at the unique *Bam*HI site, was obtained from the American Type Culture Collection (Rockville, Md.). Plasmid pYA301, bearing the *ACT1* gene (10), was provided by D. Gallwitz. Plasmid p69A, bearing the *MF*α*1* gene (19), and plasmid p334, bearing the *STE2* gene, were provided by I. Herskowitz. Plasmid 2.5, bearing the *MAT*α gene, was described previously (13). Plasmid pAM246, bearing a *RME1-lacZ* fusion gene, was provided by A. P. Mitchell.

Media and genetic and biochemical methods. The media used for cultivation of *S. cerevisiae* and *E. coli* cells were described previously (53). Sporulation medium for *S. cerevisiae* was as described previously (38). The genetic methods used for *S. cerevisiae* were as described previously (45). To construct a hybrid between two *S. cerevisiae* strains of the same mating type or between nonmating strains, the cell fusion technique with cell protoplasts (13) was employed.

TABLE 1. *S. cerevisiae* strains used

Strain	Mating type[a]	Genotype[b] or parent
Haploid		
YMH2	α*	*MATa HMLα HMRa sir3::LEU2 aar1-6 ura3-52 leu2 pho3-1 pho5-1 trp1::[TRP1 MFα 1-PHO5]*[c]
YMH36	a	*MATa ura3-52 leu2-3,112 his4 thr4*
YMH37	α*	*MATa HMLα HMRa sir3::LEU2 aar1-6 ura3-52 leu2-3,112 trp1 his5*
YMH38	Non	*MATa HMLα HMRa sir3::LEU2 ura3-52 leu2-3,112 trp1 his5*
YMH39	a	*MATa ura3-52 leu2-3,112*
YMH40	α	*MATα ura3-52 trp1*
YMH47	α	*MATα ura3-52 leu2-3,112 trp1 pho3-1 pho5-1*
YMH49	α*	*MATa HMLα HMRa sir3::LEU2 aar1-6 ura3-52 leu2 trp1 ade2 lys2 pho3-1 pho5-1*
YMH54	Non	*MATa HMLα HMRa sir3-4 aar1::LEU2 ura3-52 leu2-3,112 his4-580 pho3-1 pho5-1*
YMH55	Non	*MATα aar1::LEU2 ura3-52 leu2-3,112 trp1 pho3-1 pho5-1*
YMH56[d]	a	*MATa aar1::LEU2 ura3-52 leu2-3,112 his4 thr4 pho3-1 pho5-1*
YMH61[e]	Non	*MATa HMLα HMRa sir3::LEU2 aar1::LEU2 ura3-52 leu2-3,112 his4-580* and/or *his5 pho3-1 pho5-1*
YMH62[e]	Non	*MATa HMLα HMRa sir3::LEU2 aar1::LEU2 ura3-52 leu2-3,112 trp1 pho3-1 pho5-1*
YMH63[e]	Non	*MATa HMLα HMRa sir3::LEU2 aar1::LEU2 ura3-52 leu2-3,112 trp1 his4-580* and/or *his5 pho3-1 pho5-1*
SH2432	Non	*MATa HMLα HMRa sir3-4 ura3-52 leu2-3,112 his4-580 pho3-1 pho5-1*
SH2503	Non	*MATa HMLα HMRa sir3::LEU2 ura3-52 leu2 trp1 ade2-101* and/or *ade10 lys2-801 pho3 pho5*
SH2518	Non	*MATa HMLα HMRa sir3::LEU2 ura3 leu2 ilv1 pho3-1 pho5-1*
Diploid		
YMD28	Non	YMH39 × YMH40
YMD29[f]	Non	YMH55 + YMH56
YMD32[f]	Non	YMH61 + YMH62
YMD33[f]	α*	YMH37 + YMH49
YMD35[f]	Non	YMH38 + SH2503
SH1536	α	Diploid homozygous for *MATα trp1 leu2 his4 thr4 arg4*
SH1995	a	Diploid homozygous for *MATa leu2-3,112 trp1 his3*

[a] α* indicates the α-mating type showing weak mating with α cells. Non, nonmating type.
[b] All the strains have the *ho* genotype. Genetic symbols are as described by Mortimer and Schild (25). The disrupted alleles of *SIR3* and *AAR1* by insertion of the *LEU2* DNA, *sir3::LEU2* and *aar1::LEU2*, at the respective loci were confirmed by genomic Southern blot analysis.
[c] *trp1::[TRP1 MFα1-PHO5]* indicates the integration of the *MFα 1-PHO5* fusion gene into the *trp1* locus by using a plasmid constructed by ligating a 3.4-kbp *Bam*HI-*Pvu*II fragment of pHK104 (18) bearing the *MFα 1-PHO5* fusion gene with the *Bgl*II-*Pvu*II fragment of YRp7 bearing the *TRP1* DNA.
[d] YMH56 is a meiotic segregant from a fusion with YMH55 + YMH36.
[e] YMH61, YMH62, and YMH63 are meiotic segregants from a fusion with YMH54 + YMH38.
[f] These diploids were constructed by the cell fusion technique.

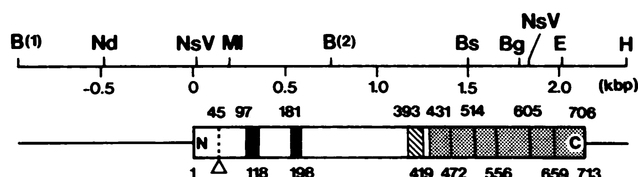

FIG. 1. Restriction map of a DNA fragment bearing the *AAR1* gene and the domain structure of the predicted AAR1 protein. The two closed boxes represent glutamine-rich domains, and the hatched box represents a serine- and threonine-rich domain. The dotted boxes indicate segmental units homologous to those in the β subunits of G proteins. The open triangle indicates the position of the N-terminal methionine of the SFL2 protein (9). Restriction sites: B, *Bam*HI; Bg, *Bgl*II; Bs, *Bst*PI; E, *Eco*RI; H, *Hin*dIII; Ml, *Mlu*I; Nd, *Nde*I; and NsV, *Nsp*(7524)V. Figures on the predicted AAR1 protein indicate the initial and ending amino acid positions of the domains.

Yeast was transformed by the method of Ito et al. (15), and *E. coli* was transformed by the method of Morrison (24). Acid phosphatase (APase; EC 3.1.3.2) activity of yeast colonies was detected by a staining method based on a diazo-coupling reaction (47). Methods for preparation and manipulation of DNAs and RNAs were as previously described (26, 45). The nucleotide sequence was determined by the dideoxy chain termination method (33).

RESULTS

Cloning, sequencing, and mapping of the *AAR1* gene. To facilitate cloning of the *AAR1* gene, we constructed an *aar1* mutant, YMH2, having the *ho MATa HMLα HMRa sir3::LEU2 aar1-6 ura3 pho3 pho5 trp1::[TRP1 MFα1-PHO5]* genotype. Though *sir3* disruption leads to expression of the *HMLα* and *HMRa* genes, YMH2 cells mated with **a** cells and also mated weakly with α cells, which is a characteristic of the **a**/α *aar1* mutant (13) (this bimating phenotype is termed α*). Expression of the *MFα1-PHO5* fusion gene is under the control of the mating type, since the APase encoded by *PHO5* of the fusion gene with the promoter of *MFα1* is expressed in α cells but not in **a** or **a**/α cells. Therefore, colonies of YMH2 with chromosomal integration of the *MFα1-PHO5* fusion gene were red when stained for APase activity, because the *aar1* mutation allows expression of the *α1* cistron (13) and consequently expression of *MFα1*, an α-specific gene. By selecting transformants of YMH2, with DNAs of the gene library CEN BANK A, showing the Ura$^+$, white on APase staining, and nonmating phenotypes, we isolated a plasmid, pYMC5, with a 10.2-kbp insert in the *Bam*HI site of YCp50.

We confirmed that the 10.2-kbp insert of pYMC5 contains the *AAR1* gene by insertion of a 13.4-kbp *Pvu*I-*Sma*I fragment of pYMC5 bearing the 10.2-kbp fragment into a chromosome of the *aar1* mutant YMH2 after connecting it in the *Pvu*I-*Sma*I gap of YIp5 and linearizing the resultant chimeric plasmid by digestion with *Hpa*I or *Xho*I. [The *Hpa*I and *Xho*I sites are in the 10.2-kbp fragment but outside the 3.3-kbp *Bam*HI(1)-*Hin*dIII region (Fig. 1) at positions ca. 3.5 kbp and 3 kbp, respectively, from the *Hin*dIII end.] Each Ura$^+$ transformant with the *Hpa*I- or *Xho*I-restricted DNA was fused with *S. cerevisiae* cells of strain SH2518 (*MATa HMLα HMRa sir3 AAR1$^+$ ura3*). The resultant two diploids were sporulated and dissected. All the tetrad segregants from 16 asci in each diploid showed the nonmating phenotype, indicating that the plasmid DNA was integrated at or near the *aar1-6* mutant locus of YMH2 and that the 10.2-kbp fragment contained the *AAR1* gene.

The essential region for complementation of the α* mating type of YMH2 was delimited into a 3.3-kbp *Bam*HI(1)-*Hin*dIII fragment (Fig. 1) by subcloning various restriction fragments of the 10.2-kbp fragment into YCp50. This 3.3-kbp

TABLE 2. Sequence deviations in *AAR1*, *TUP1*, and *SFL2* DNAs[a]

Nucleotide position in *TUP1*		Deviation in:		
Serial no.	No. relative to ORF	*TUP1*	*AAR1/AER2*	*SFL2*
8	−501	C	Δ	
9	−500	C	A	
10	−499	C	A	
129	−380	C	A	
182	−327	T	Δ	
194	−315	T	Δ	
200	−309	C	T	
471	−38	C	C	G
542	+34	C	C	Δ (frameshift)
697	+189	G (Glu)	A (Glu)	A (Glu)
732	+224	C (Ala)	A (Glu)	A (Glu)
807	+299	A (Gln)	G (Arg)	G (Arg)
856	+348	A (Gln)	G (Gln)	G (Gln)
1630	+1122	C (Asp)	T (Asp)	T (Asp)
1649	+1141	T (Leu)	C (Leu)	C (Leu)
2263	+1755	G (Thr)	T (Thr)	T (Thr)
2561	+2053	T (Ser)	C (Pro)	C (Pro)
2833	+2324 (3′ downstream)	T	TTT	TTT

[a] Nucleotide and amino acid substitutions observed in *AAR1/AER2* (54) and *SFL2* (9) genes and proteins deduced from DNA sequences are listed in comparison with those of *TUP1* DNA (51). Δ, deletion.

region was then sequenced. We found that this region contained a single open reading frame (ORF) of 2,139 bp, consisting of 713 amino acid codons and capable of encoding a protein with a calculated molecular mass of 78 kDa (details not shown). The nucleotide sequence of *AAR1*, which has so far been read from nucleotide position −945 in the upstream region of the ORF to 244 bp beyond the C-terminal codon, has the same nucleotide sequence (with minor differences) as those reported for *TUP1* (51), which is involved in thymidine uptake, and *SFL2* (9), a suppressor gene for flocculation, and exactly the same sequence as that of *AER2* (54), which is involved in the control of heme-regulated and catabolite-repressed genes (Table 2). The identities of the *AAR1* and *TUP1* genes are also supported by the finding that the *AAR1* locus was mapped at a site 35 centimorgans distal from the *MAT* locus on the right arm of chromosome III by conventional tetrad analysis of 95 asci of an *ura3/ura3* homozygous diploid which was heterozygous for the wild-type *AAR1*$^+$ gene and the same *AAR1*$^+$ gene but tagged with the *URA3*$^+$ gene (details not shown).

The nucleotide sequence of *AAR1/AER2*, however, resembles that of *SFL2* DNA rather than that of *TUP1* DNA, and the products of the coding regions of *AAR1/AER2* and *SFL2* have the same deduced amino acid sequences, whereas the *SFL2* ORF consists of 669 amino acid codons. This is due to deletion of a C residue at nucleotide position +34 in the *SFL2* DNA relative to the translation initiation codon of the *AAR1/TUP1/AER2* sequence. This deletion results in initiation of the *SFL2* ORF at nucleotide position +133 of the *AAR1/TUP1/AER2* sequence. Consequently, the SFL2 protein should have 44 fewer amino acid residues than the AAR1/TUP1/AER2 protein at its N terminus.

To see whether the initiation codon of *ARR1* starts at nucleotide position +1 or +133, we used two YCp50-based plasmids bearing the 3.3-kbp *Bam*HI(1)-*Hin*dIII fragment of the *AAR1* DNA. One of the plasmids obtained by digestion, filling in, and religation had a frameshift mutation at the

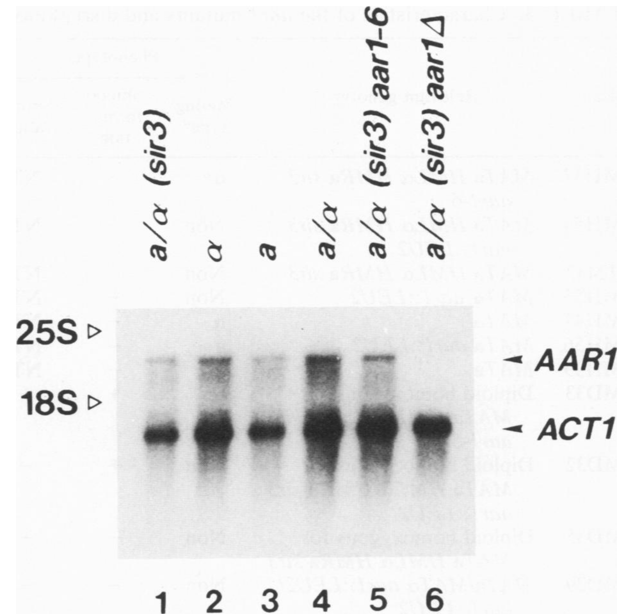

FIG. 2. Transcription of the *AAR1* gene under various conditions of mating-type information. Poly(A)-enriched RNA was prepared from strain YMH38 (*MAT***a** *HML*α *HMR***a** *sir3*) (lane 1), YMH47 (*MAT*α) (lane 2), YMH39 (*MAT***a**) (lane 3), YMD28 (*MAT***a**/*MAT*α) (lane 4), YMH37 (*MAT***a** *HML*α *HMR***a** *sir3 aar1-6*) (lane 5), and YMH63 (*MAT***a** *HML*α *HMR***a** *sir3 aar1::LEU2*) (lane 6). Samples of 2 μg of poly(A) RNA were applied to slots in an agarose (1.5%) gel containing formaldehyde, and the samples were separated by electrophoresis. The gel was subjected to Northern blot hybridization with a mixture of the ^{32}P-labeled 1.0-kbp *Bam*HI-*Bgl*II fragment bearing the *AAR1* gene (Fig. 1), prepared from plasmid pYMC5, and the 1.0-kbp *Hin*dIII-*Xho*I fragment bearing the *ACT1* gene, prepared from plasmid pYA301, as probes. The radioactivities of the probes were both adjusted to 1.0 × 10^8 cpm per μg of DNA. 25S and 18S indicate the migration positions of the respective rRNAs of *S. cerevisiae* used as size markers (29).

Nsp(7524)V site in the *AAR1* ORF at nucleotide position +15, which is outside the *SFL2* ORF. Another plasmid was constructed similarly, by modification of the *Mlu*I site (position +212) located in both the *AAR1* and *SFL2* ORFs. Neither plasmid was able to complement the *aar1* mutation of YMH2, indicating that the *Nsp*(7524)V site is in the protein coding region.

Detection of *AAR1* mRNA. To determine whether expression of the *AAR1* gene is under the control of mating-type information, we prepared poly(A)-enriched RNAs from cells of various genotypes for the mating-type information and for the *AAR1* gene. These poly(A) RNA samples were subjected to Northern (RNA) blot hybridization with a ^{32}P-labeled 1.0-kbp *Bam*HI(2)-*Bgl*II fragment of the *AAR1* DNA (Fig. 1) as a probe (Fig. 2). A single hybridization band of 2.5 kb was observed in all these strains, except the *aar1* gene disruptant. The same transcript was estimated to be 2.6 kb by Williams and Trumbly (51). No significant differences were found in the amounts of the *AAR1* transcripts, irrespective of the differences of the mating-type information, with the *ACT1* transcript as a standard. The amount of the *AAR1* transcript was much less than that of the *ACT1* transcript, judging from the hybridization signals. These results indicate that *AAR1* transcription is low and not regulated by mating-type information and suggest that the gene might be tran-

TABLE 3. Characteristics of the *aar1* mutants and disruptants

Strain	Relevant genotype	Phenotype		
		Mating type[a]	Shmoo formation	Sporulation
YMH37	*MAT***a** *HML*α *HMR***a** *sir3 aar1-6*	α*	+	NT[b]
YMH54	*MAT***a** *HML*α *HMR***a** *sir3 aar1::LEU2*	Non	+	NT
SH2432	*MAT***a** *HML*α *HMR***a** *sir3*	Non	−	NT
YMH55	*MAT*α *aar1::LEU2*	Non	+	NT
YMH47	*MAT*α	α	−	NT
YMH56	*MAT***a** *aar1::LEU2*	**a**	−	NT
YMH39	*MAT***a**	**a**	−	NT
YMD33	Diploid homozygous for *MAT***a** *HML*α *HMR***a** *sir3 aar1-6*	α*	+	+
YMD32	Diploid homozygous for *MAT***a** *HML*α *HMR***a** *sir3 aar1::LEU2*	Non	+	−
YMD35	Diploid homozygous for *MAT***a** *HML*α *HMR***a** *sir3*	Non	−	+
YMD29	*MAT***a**/*MAT*α *aar1::LEU2*/*aar1::LEU2*	Non	−	−
YMD28	*MAT***a**/*MAT*α	Non	−	+
SH1536	*MAT*α/*MAT*α	α	−	−
SH1995	*MAT***a**/*MAT***a**	**a**	−	−

[a] Non, nonmating phenotype.
[b] NT, not tested.

scribed constitutively. Constitutive transcription of the *AAR1* gene is consistent with the fact that it has a poly(dA-dT) stretch in the upstream region of the ORF, from nucleotide position −45 to −71, a structure that has been suggested to cause constitutive transcription of a gene (43).

Effects of *AAR1* gene disruption. The chromosomal *AAR1* gene was disrupted to investigate its function. A 3.7-kbp *Hin*dIII fragment, consisting of a 2.7-kbp *Bgl*II fragment of the *LEU2* DNA prepared from YEp13 which was sandwiched between the 0.46-kbp *Bam*HI(1) (changed to *Hin*dIII)-*Nde*I (changed to *Bgl*II) and 0.59-kbp *Bgl*II-*Hin*dIII fragments of the *AAR1* DNA (these two fragments constituted the end regions of the 3.3-kbp *AAR1* fragment [Fig. 1]), was prepared and was inserted into a chromosome of SH2432 (*MAT***a** *HML*α *HMR***a** *sir3 leu2 AAR1*$^+$) and YMH47 (*MAT*α *leu2 AAR1*$^+$) in place of the wild-type *AAR1* gene. The Leu$^+$ transformant strain of SH2432, YMH54 (*MAT***a** *HML*α *HMR***a** *sir3 aar1::LEU2*), had the nonmating phenotype (Table 3). This finding was in contrast with the fact that the original *aar1* mutants isolated by in vivo mutagenesis (13) exhibited the α* mating type. A Leu$^+$ transformant of YMH47, strain YMH55 (*MAT*α *aar1::LEU2*), also showed the nonmating phenotype, whereas strain YMH56 (*MAT***a** *aar1::LEU2*), a meiotic segregant from a diploid obtained by YMH55 + YMH36 cell fusion, showed the **a** mating potency because this strain had *MAT***a**. In addition to the nonmating phenotype of the *aar1* disruptants, we found that α and **a**/α cells with an *aar1* mutation, whether it was a disrupted allele or a putative point mutation by in vivo mutagenesis, formed shmoos as if there were mating-competent cells secreting α- or **a**-pheromone present, as described for the *tup1* mutant (21). These facts suggest that α and **a**/α cells with *aar1* mutations express both α- and **a**-specific genes and further suggest that the *AAR1* gene product is required not only for **a**1-α2 repression of the *α1* cistron, as observed previously (13), but also for α2 repression of the **a**-specific genes.

Transcription of the α- and a-specific genes in *aar1* mutants. The possibility that the *aar1* mutants express the α- and **a**-specific genes was confirmed by Northern blot hybridization. The *MF*α*1* gene (an α-specific gene [14]) and the *STE2* gene (an **a**-specific gene [14]) were not transcribed in **a**/α *AAR1*$^+$ cells but were transcribed in the *aar1* mutant having α and **a**/α information, and the *MF*α*1* gene was not transcribed in the **a**-mating-type cells, as expected (Fig. 3A). A trace amount of *STE2* transcript is always detected in α *AAR1*$^+$ cells in our strain (Fig. 3A, lane 5) for some unknown reason. The transcription of *STE2* in α and **a**/α cells having the disrupted *aar1* gene clearly indicated that the *AAR1* gene product is also required for α2 repression, but the reasons for the low levels of transcriptions of *STE2* and *MF*α*1* in the α *aar1* disruptant are unknown. The *α1* cistron was also transcribed in the **a**/α *aar1* mutant cells (Fig. 3B), as previously described (13).

Since the *mat*α*2* mutation is known to confer the nonmating phenotype by expression of both the α- and **a**-specific genes (40, 42, 52), by using Northern analysis, we examined whether the *aar1* mutation inhibits transcription of the α2 cistron. However, we detected the α2 transcript in all the α and **a**/α cells of *aar1* mutants examined (Fig. 3C), as in *AAR1*$^+$ cells, indicating that the *aar1* mutation does not affect transcription of the α2 cistron. Although the **a**2 transcript is not detectable in *MAT***a** cells in Fig. 3C (lanes 6 and 7), it was detected in lanes of the *aar1* disruptant and *AAR1*$^+$ cells after overexposure of the emulsion (data not shown). We also detected **a***1* transcripts in all **a** and **a**/α cells of the *aar1* mutants examined (data not shown). Thus, we conclude that the *AAR1* gene is not involved in transcription of the *MAT* genes but that the *AAR1* gene product is essential for function of the mating-type gene products.

Transcription of the *RME1* gene in *aar1*/*aar1* diploids. The *RME1* gene, encoding the negative regulator for sporulation, is subject to **a**1-α2 repression. Therefore, expression of *RME1* is repressed in **a**/α diploids and the cells are able to sporulate (23). Previously, we reported that the *AAR1* gene product is not concerned with repression of the *RME1* gene, because we found that an **a**/α diploid that was homozygous for the *aar1* mutation could still sporulate (13). However, we found that **a**/α diploids that were homozygous for the disrupted *aar1* gene, strains YMD29 and YMD32, could not sporulate (Table 3). This finding strongly suggests that the *AAR1* gene product is also indispensable for the **a**1-α2 repression of *RME1*. To confirm this, we examined the expression of the transcript of the *RME1* gene in diploids that were homozygous for the *aar1-6* mutant allele or the disrupted *aar1* gene. The *RME1* transcript was detected in all the diploids homozygous for the *aar1* mutation, such as the **a**/**a** and α/α diploids, but was markedly repressed in **a**/α *AAR1*$^+$ cells (Fig. 3B). However, we noticed that the transcriptional level of the *RME1* gene in the *aar1-6* mutant was somewhat lower than that in the *aar1* disruptant. These observations suggest that sporulation occurs if transcription of the *RME1* transcript decreases below a certain level, as in the *aar1-6* mutant. (The reason for the double-band hybridization signals close to the *α1* transcript of the **a**/α *AAR1*$^+$ cells [Fig. 3B] is unknown.)

DISCUSSION

In the previous study, we observed that **a**/α cells with the *aar1* mutation showed a characteristic α* phenotype and concluded that the AAR1 protein is required for the **a**1-α2 repression only in the transcription of the *α1*-cistron and

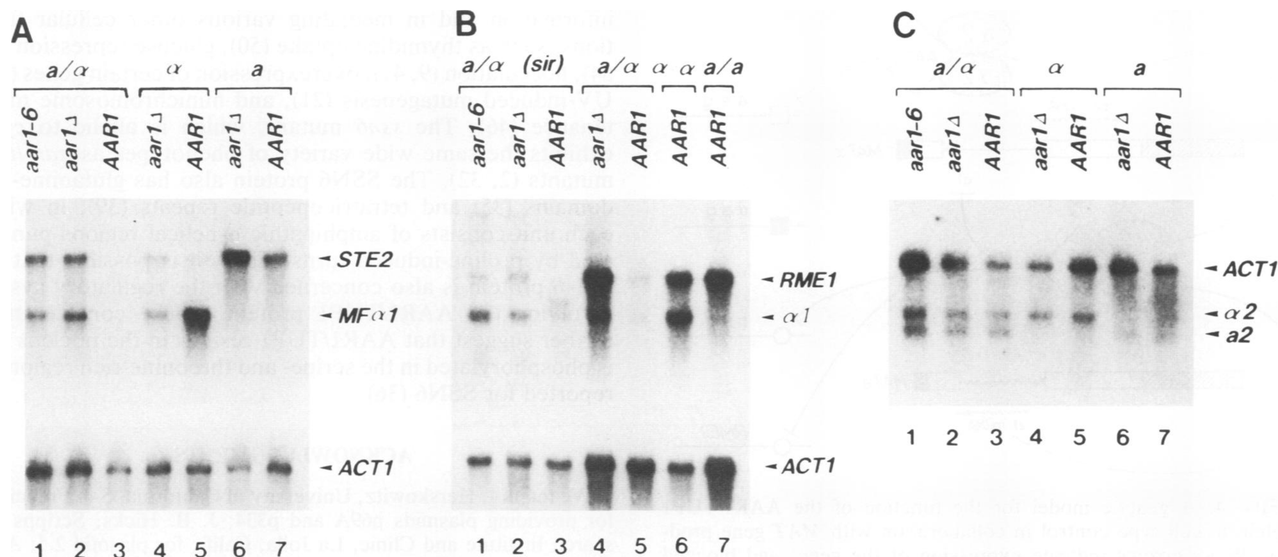

FIG. 3. Effects of the *aar1* mutation on transcription of the cell-type-specific genes. Poly(A)-enriched RNA was fractionated by agarose (1.5%) gel electrophoresis (2 μg per lane) in the presence of formaldehyde and transferred to a nylon filter. (A) RNA samples were prepared from strain YMH37 (*MAT***a** *HML*α *HMR***a** *sir3 aar1-6*) (lane 1), YMH63 (*MAT***a** *HML*α *HMR***a** *sir3 aar1*::*LEU2*) (lane 2), YMH38 (*MAT***a** *HML*α *HMR***a** *sir3*) (lane 3), YMH55 (*MAT*α *aar1*::*LEU2*) (lane 4), YMH47 (*MAT*α) (lane 5), YMH56 (*MAT***a** *aar1*::*LEU2*) (lane 6), and YMH39 (*MAT***a**) (lane 7). The RNA blots were hybridized with a mixture of ^{32}P-labeled 1.4-kbp *Eco*RI-*Sal*I fragment bearing the *MF*α*1* gene from plasmid p69A and a 1.3-kbp *Hin*dIII-*Pst*I fragment bearing the *STE2* gene from plasmid p334 as probes and rehybridized with a ^{32}P-labeled 1.0-kbp *Hin*dIII-*Xho*I fragment carrying the *ACT1* gene from plasmid pYA301 as an internal marker. (B) Poly(A)-enriched RNA samples were prepared from strains YMD33 (diploid homozygous for *MAT***a** *HML*α *HMR***a** *sir3 aar1-6*) (lane 1), YMD32 (diploid homozygous for *MAT***a** *HML*α *HMR***a** *sir3 aar1*::*LEU2*) (lane 2), YMD35 (diploid homozygous for *MAT***a** *HML*α *HMR***a** *sir3*) (lane 3), YMD29 (*MAT***a**/*MAT*α *aar1*::*LEU2*/*aar1*::*LEU2*) (lane 4), YMD28 (*MAT***a**/*MAT*α) (lane 5), SH1536 (*MAT*α/*MAT*α) (lane 6), and SH1995 (*MAT***a**/*MAT***a**) (lane 7). The RNA samples were blotted and hybridized with a mixture of a ^{32}P-labeled 0.8-kbp *Eco*RI fragment bearing the *RME1* gene (**a**/α-specific gene) from plasmid pAM246 and a 1.1-kbp *Eco*RV fragment bearing the α*1* cistron from plasmid 2.5 as probes and rehybridized with the ^{32}P-labeled *ACT1* fragment as an internal marker. (C) The same RNA samples as in panel A were blotted from another gel and hybridized with a ^{32}P-labeled 0.6-kbp *Nde*I-*Xba*I fragment bearing the α*2* cistron from plasmid 2.5 and the *ACT1* fragment. The **a***2* transcript was probed with the α*2* cistron DNA, because the α*2* and **a***2* cistrons have a common DNA sequence (17). The radioactivities of the probes were adjusted to 1.0×10^8 cpm per μg of DNA.

haploid-specific genes and not for **a**1-α2 repression of *RME1* or α2 repression of **a**-specific genes (13). However, in the present study, we found that **a**/α cells with a disrupted *aar1* gene had the nonmating phenotype because of the expression of both the **a**- and α-specific genes (Fig. 3) and were unable to sporulate (Table 3). Thus, the *AAR1* gene is also required for both α2 repression and **a**1-α2 repression of *RME1*. The nonmating phenotype has also been observed in α cells with a *tup1* mutation (50), as in cells with a disrupted *aar1* gene. This is in contrast to the nonmating phenotype of wild-type **a**/α cells, which is due to the absence of expression of both the **a**- and α-specific genes.

The discrepancy between the observations of the α* mating type in the *aar1* mutants by in vivo mutagenesis and the nonmating type in the *aar1* disruptants might be because the in vivo mutants were selected from **a**/α cells by picking colonies showing the α mating type (13). Therefore, the in vivo mutants probably produced a mutant AAR1 protein, and their α2 repression should be active, even if partial, whereas no AAR1 protein was produced in the *aar1* disruptants. Diploid cells homozygous for the *aar1* in vivo mutation could sporulate, whereas the *aar1* disruptants could not. This might also be explained by supposing that the *aar1* mutant protein of the in vivo mutants was not fully inactivated and repressed *RME1* transcription, at least partially, whereas the *aar1* disruption resulted in full expression of *RME1* (Fig. 3B), turning off the sporulation circuit.

Several explanations of the function of AAR1/TUP1 protein in the mechanisms of **a**1-α2 and α2 repression are possible. One possibility is that it catalyzes the conversions of the **a**1 and α2 proteins to their active forms. However, no amino acid sequences conserved in, for example, protein kinases or protein phosphatases were found in the amino acid sequence deduced from the ORF of *AAR1*/*TUP1*. The **a**1 and α2 polypeptides synthesized in vitro could bind with the operator site of the α*1* cistron only when they were cotranslated (6). This finding suggests another possibility, that the AAR1/TUP1 protein may act as an enzyme to form active heterodimers, and also possibly active homodimers, of **a**1 and α2 polypeptides by catalyzing, for example, disulfide bond formation between cysteine residues of the polypeptides, as suggested for the α2 polypeptide by Sauer et al. (34). Another possibility is that the AAR1/TUP1 protein inhibits α2 protein degradation, even though the stabilities of α2 transcripts were the same in the *aar1* mutants and the *AAR1*$^+$ cells (Fig. 3C).

However, we think that the most plausible role of the AAR1/TUP1 protein is direct involvement in repressor complexes for repression of both **a**1-α2 and α2. The amino acid sequence of the predicted AAR1/TUP1 protein supports this idea, since it has significant similarity with the β subunit of G proteins in its C-terminal half, in two glutamine-rich regions in its N-terminal half, and in the serine- and threonine-rich region in the middle (51, 54). The G proteins have a complex structure with three different subunits, α, β, and γ, and function in various signal transduction pathways (11). Glu-

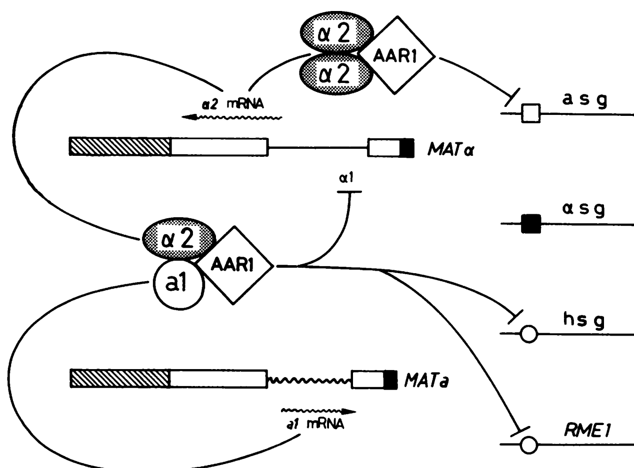

FIG. 4. A genetic model for the function of the AAR1/TUP1 protein in cell type control in collaboration with *MAT* gene products. Wavy arrows indicate expression of the gene, and blocked lines represent repression of the genes. Abbreviations: αsg, α-specific genes; asg, **a**-specific genes; hsg, haploid-specific genes.

tamine-rich regions were found in the *opa* box of *Drosophila melanogaster* (20, 31, 49) and in several transcriptional regulators in *S. cerevisiae* (28, 30, 35, 44). The glutamine-rich domains in human transcription factor Spl were suggested to play a role in protein-protein interaction (4). Serine- and threonine-rich regions have been suggested to be active sites of posttranslational modification or to act as hydrogen bond acceptors and donors (5). All these lines of evidence strongly suggest that the AAR1/TUP1 protein acts in complexes with other proteins, possibly with α2 and **a**1 polypeptides, and exerts its function as a component of transcriptional repressors in α2 repression and **a**1-α2 repression in cell type control (Fig. 4).

In connection with the above idea, the PRTF (1)/GRM protein (16), encoded by the *MCM1* (28) gene, is particularly interesting. This protein has glutamine-rich domains (28) and a DNA-binding domain specific for the P box in the upstream regions of α- and **a**-specific genes (1). It acts as a transcriptional regulator by interacting with both the α1 and α2 proteins. A mutation in the PRTF/GRM/MCM1 protein conferred sterility only on *MAT*α cells, a phenotype similar to *aar1*-disrupted cells. STE12 also binds to the pheromone response element (PRE) located close to the P box in the 5'-upstream regions of various genes, including α- and **a**-specific genes and the Tyl element genes (7). Thus, the repressor complexes involving the AAR1/TUP1 protein may interact with PRTF/GRM/MCM1, and possibly with the STE12 protein, to repress the transcription of genes for cell type control, of Tyl elements, and of other genes.

By analysis of deletion mutants of the α2 polypeptide, Hall and Johnson (12) showed that the domains required for **a**1-α2 and α2 repression are separate. We have also isolated missense mutants of α2 polypeptide showing defective **a**1-α2 repression but normal α2 repression (13). Probably, the AAR1/TUP1 polypeptide also has separate domains for **a**1-α2 and α2 repression, and in vivo *aar1* mutants may have a mutation in the domain for **a**1-α2 repression but an intact domain for α2 repression.

These considerations strongly suggest that the AAR1/TUP1 protein is a key factor in mediating the control of cell type by mating-type information and possibly by other information and in mediating various other cellular functions, such as thymidine uptake (50), glucose repression (41, 54), flocculation (9, 41), overexpression of certain genes (32), UV-induced mutagenesis (21), and minichromosome maintenance (46). The *ssn6* mutant, which is allelic to *cyc8*, exhibits the same wide variety of phenotypes as *aar1/tup1* mutants (2, 32). The SSN6 protein also has glutamine-rich domains (35) and tetratricopeptide repeats (39), in which each unit consists of amphipathic α-helical regions punctuated by proline-induced turns. Thus, it is possible that the SSN6 protein is also concerned with the regulatory system involving the AAR1/TUP1 protein. These considerations further suggest that AAR1/TUP1 resides in the nucleus and is phosphorylated in the serine- and threonine-rich region, as reported for SSN6 (36).

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