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The Yeast *ARD1* Gene Product Is Required for Repression of Cryptic Mating-Type Information at the *HML* Locus†

MALCOLM WHITEWAY,[1]‡ RICHARD FREEDMAN,[2]§ SCOTT VAN ARSDELL,[2] JACK W. SZOSTAK,[1] AND JEREMY THORNER[2]*

Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114,[1] and Department of Biochemistry, University of California, Berkeley, California 94720[2]

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Mutations in the *ARD1* gene prevent yeast cells from displaying G1-specific growth arrest in response to nitrogen deprivation and cause *MAT*a haploids (but not *MAT*α haploids) to be mating defective. Analysis of cell type-specific gene expression by examination of RNA transcripts and measurement of β-galactosidase activity from yeast gene-*lacZ* fusions demonstrated that the mating defect of *MAT*a *ard1* mutants was due to an inability to express genes required by *MAT*a cells for the mating process. The lack of mating-specific gene expression in *MAT*a cells was found to be due solely to derepression of the normally silent α information at the *HML* locus. The cryptic a information at the *HMR* locus was only very slightly derepressed in *ard1* mutants, to a level insufficient to affect the mating efficiency of *MAT*α cells. The preferential elevation of expression from *HML* over *HMR* was also observed in *ard1* mutants which contained the alternate arrangement of a information at *HML* and α information at *HMR*. Hence, the effect of the *ard1* mutation was position specific (rather than information specific). Although the phenotype of *ard1* mutants resembled that of cells with mutations in the *SIR1* gene, both genetic and biochemical findings indicated that *ARD1* control of *HML* expression was independent of the regulation imposed by *SIR1* and the other *SIR* genes. These results suggest that the *ARD1* gene encodes a protein product that acts, directly or indirectly, at the *HML* locus to repress its expression and, by analogy, may control expression of other genes involved in monitoring nutritional conditions.

TABLE 1. *S. cerevisiae* strains used

Strain	Genotype	Reference or source
T8-1A	*MAT***a** *his3-11,15 leu2-3,112 can1 ard1*::*HIS3*	58
T8-1B	*MAT*α *his3-11,15 leu2-3,112 can1 ard1*::*HIS3*	58
T8-1C	*MAT***a** *his3-11,15 leu2-3,112 can1 ARD1*	58
T8-1D	*MAT*α *his3-11,15 leu2-3,112 can1 ARD1*	58
T8	*MAT***a**/*MAT*α *his3-11,15*/*his3-11,15 leu2-3,112*/*leu2-3,112 can1*/*can1 ard1*::*HIS3*/*ARD1*	This work
T8-1A4	Δ*mat*::*LEU2 his3-11,15 leu2-3,112 can1 ard1*::*HIS3*	This work
T8-1C2	Δ*mat*::*LEU2 his3-11,15 leu2-3,112 can1 ARD1*	This work
DM108	*MAT***a**/*MAT*α *his3*/*his3 leu2*/*LEU2 ura3*/*ura3 trp1*/*trp1 ard1*::*HIS3*/*ARD1*	This work
DM109	*MAT***a**/*MAT*α *his3*/*his3 leu2*/*LEU2 ura3*/*ura3 trp1*/*TRP1* Δ*ard1*::*URA3*/*ARD1*	This work
K77	*MAT***a** *his3 his4 leu2-3,112 ura3-52 trp1 ste6-lacZ*	59
848	*MAT***a** *HML***a** *HMR***a** *his4 leu2 ade6 met cry1*	S. Fields
23α	*MAT*α *his4 leu2 ura3 trp1*	55
23α182	*mat*α*2 his4 leu2 ura3 trp1*	55
23α113	*mat*α*1 his4 leu2 ura3 trp1*	55
T23	*MAT*α *his4 leu2 ura3 trp1 ard1*::*URA3*	This work
T182	*mat*α*2 his4 leu2 ura3 trp1 ard1*::*URA3*	This work
T113	*mat*α*1 his4 leu2 ura3 trp1 ard1*::*URA3*	This work
J30	*MAT*α *HML***a** *HMR*α *his suc gal1 MAL*	A. Klar
K397	*MAT***a** *hml*α*-2*::*LEU2 his leu2 thr4 mal*	A. Klar
DA2102	*MAT*α *his4-519 leu2-3,112 ura3-52 suc2-*Δ*9 lys2*	3
YNN217	*MAT***a** *his3-*Δ*200 ura3-52 ade2-101*(Oc) *lys2-801*(Am)	C. Mann
YNNT8	*MAT***a**/*MAT*α *his3-*Δ*200*/*his3-11,15 leu2-3,112*/*LEU2 ura3-52*/*URA3 ade2-101*(Oc)/*ADE2 lys2-801*(Am)/*LYS2 can1*/*CAN1 ard1*::*HIS3*/*ARD1*	This work
RFY2	*MAT*α *his3 leu2 ura3 ard1*::*HIS3*	This work
RFY3	*MAT***a** *hml*α*-2*::*LEU2 leu2 ura3-52 suc2 thr4 lys2*	This work
RFY4	*MAT***a** *hml*α*-2*::*LEU2 his3 leu2 ura3 suc2 thr4*	This work
RFY10	*MAT***a** *hml*α*-2*::*LEU2 his3 leu2 ura3 ard1*::*HIS3*	This work
T1724	*MAT*α *HMR***a**::*HIS3*,*LEU2*,*URA3 his3 leu2 ura3 trp1 can1 tcm1*	A. Murray
M142.2-4B	*MAT*α *hml*α*-2*::*LEU2 HMR***a**::*HIS3*,*LEU2*,*URA3 his3 leu2 ura3 trp1*	This work
MW2A	*MAT***a** *HML***a** *HMR*α *leu2 ura3 ard1*::*URA3*	This work
MW2B	*MAT*α *HML***a** *HMR*α *leu2 ura3*	This work
MW2C	*MAT*α *HML***a** *HMR*α *leu2 ura3 ard1*::*URA3*	This work
MW2D	*MAT***a** *HML***a** *HMR*α *leu2 ura3*	This work
MW2B0K	*MAT*α *HML***a** *HMR*α *leu2 ura3 lys2*	This work
MW2C0K	*MAT*α *HML***a** *HMR*α *leu2 ura3 lys2 ard1*::*URA3*	This work
MW2B1K	Δ*mat*::*LEU2 HML***a** *HMR*α *leu2 ura3 lys2*	This work
MW2C1K	Δ*mat*::*LEU2 HML***a** *HMR*α *leu2 ura3 lys2 ard1*::*URA3*	This work
DC14	*MAT***a** *his1*	J. Hicks
DC17	*MAT*α *his1*	J. Hicks
XMB4-12B	*MAT***a** *arg9 ilv3 ura1 sst1* [*KIL*$^+$]	L. Blair
RC757	*MAT*α *his6 met1 can1 cyh1 sst2-1*	R. Chan
T8-1A0K	*lys2* derivative of T8-1A	This work
T8-1B0K	*lys2* derivative of T8-1B	This work
T8-1C0K	*lys2* derivative of T8-1C	This work
T8-1D0K	*lys2* derivative of T8-1D	This work
T8-1A4K	*lys2* derivative of T8-1A4	This work
T8-1C2K	*lys2* derivative of T8-1C2	This work
T109.6-3C	*MAT***a** *his3 leu2 ura3*	This work
T109.6-2B	*MAT***a** *his3 leu2 ura3* Δ*ard1*::*URA3*	This work
T109.6-3A	*MAT*α *his3 leu2 ura3*	This work
T109.6-4D	*MAT*α *his3 leu2 ura3* Δ*ard1*::*URA3*	This work
DM126	Diploid from cross RFY4 × T109.6-4D	This work

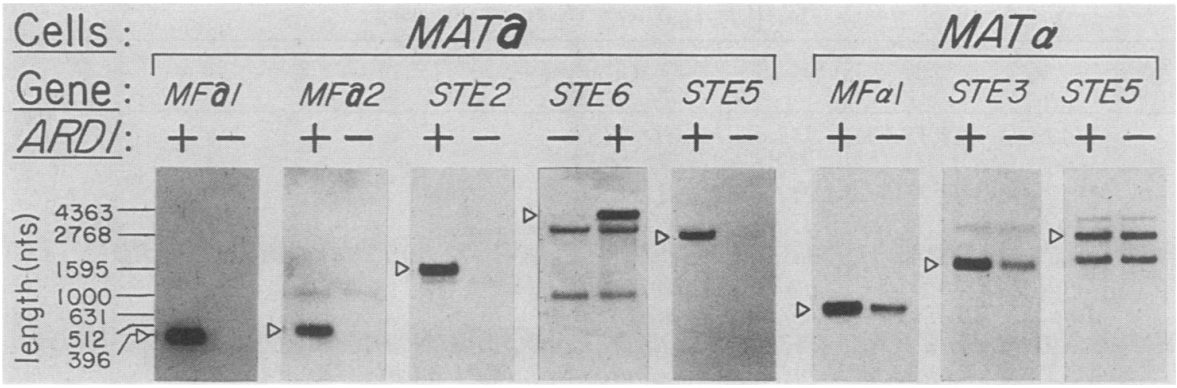

FIG. 1. Effect of an *ard1* mutation on expression of cell type-specific genes. Poly(A)$^+$ RNA was prepared from strains T8-1C (*MAT***a** *ARD1*), T8-1A (*MAT***a** *ard1*), T8-1D (*MATα ARD1*), and T8-1B (*MATα ard1*), subjected to gel electrophoresis, blotted, and hybridized to radioactive probes derived from the cloned genes indicated, all as described in Materials and Methods. nts, Nucleotides. Arrowheads indicate transcripts of the genes specified.

TABLE 2. Effect of an *ard1* mutation on *STE6* and *HO* expression

Gene fusion or plasmid and strain	Genotype		β-Galactosidase activity[a] (U)
	MAT	*ARD1*	
ste6-lacZ[b]			
M95-16D	**a**	+	6.83 ± 0.08
M95-18B	**a**	+	6.53 ± 0.33
M95-19D	**a**	+	7.25 ± 0.25
M95-20D	**a**	+	6.55 ± 0.05
M95-23C	**a**	+	7.93 ± 0.28
M95-21A	**a**	−	0.90 ± 0.10
M95-21D	**a**	−	0.55 ± 0.05
M95-16A	α	+	0.15 ± 0.05
pste6-lacZ[c]			
T86-24C	**a**	+	63.00 ± 2.00
T86-30C	**a**	+	69.00 ± 3.00
T86-24B	**a**	−	0.86 ± 0.37
T86-30B	**a**	−	1.60 ± 0.40
T86-24D	α	+	3.10 ± 1.23
T86-30A	α	+	0.15 ± 0.01
T86-24A	α	−	0.10 ± 0.05
T86-30D	α	−	0.22 ± 0.06
pho-lacZ[d]			
T108-2C	**a**	+	0.77 ± 0.10
T108-4C	**a**	+	0.57 ± 0.10
T108-2B	**a**	−	0.18 ± 0.02
T108-4A	**a**	−	0.14 ± 0.02
T108-2D	α	+	0.76 ± 0.14
T108-4D	α	+	1.05 ± 0.15
T108-2A	α	−	0.65 ± 0.06
T108-4B	α	−	0.77 ± 0.06

[a] Activity was calculated in Miller units (45), and values presented are the averages and ranges of duplicate determinations.

[b] Congenic strains derived from a cross of K77 to T8-1B, as described in Materials and Methods.

[c] Isogenic strains derived from a diploid strain (T8) transformed with plasmid p61-δ31-1, as described in Materials and Methods.

[d] Isogenic strains derived from a diploid strain (DM108) transformed with plasmid p50-12-1871, as described in Materials and Methods.

TABLE 3. Effect of an *ard1* mutation on expression of a heat shock promoter

Strain[a]	Genotype		β-Galactosidase activity[b] (U)	
	MAT	*ARD1*	23°C	37°C
T870-10D	**a**	+	4.9	255.0
T870-13A	**a**	+	5.1	288.0
T870-10A	**a**	−	5.4	147.0
T870-13B	**a**	−	3.6	149.0
T870-10B	α	+	7.0	251.0
T870-13D	α	+	3.2	241.0
T870-10C	α	−	2.8	123.0
T870-13C	α	−	4.7	177.0

[a] Isogenic strains derived from a diploid strain (T8) transformed with plasmid pIT210 (*hsp70-lacZ*), as described in Materials and Methods.

[b] Cultures were subjected to temperature shift as described in Materials and Methods prior to assay of β-galactosidase activity. Values represent the results of single determinations in Miller units (45).

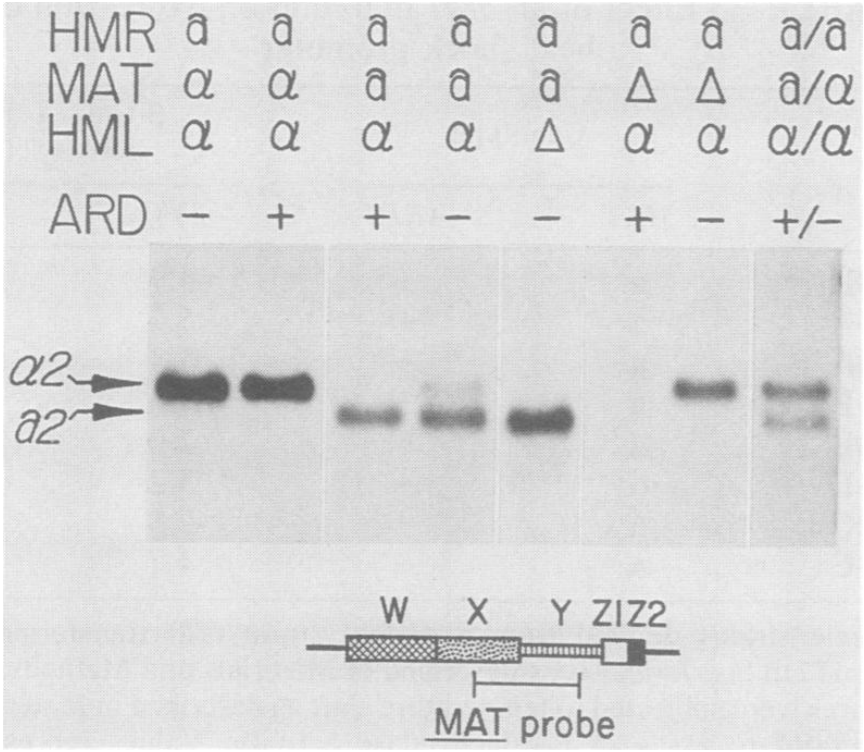

FIG. 2. Effect of an *ard1* mutation and the cryptic mating type loci on transcription of the mating type genes. Poly(A)$^+$ RNA was prepared from the following strains (with the relevant genetic constitution indicated): (left to right) T8-1B, T8-1D, T8-1C, T8-1A, RFY10, T8-1C2, T8-1A4, and YNNT8, subjected to gel electrophoresis, blotted, and hybridized to a radioactive probe (pDx2-18) specific for detecting transcripts from the region of *MAT* indicated.

TABLE 4. Effect of an *ard1* mutation and the cryptic mating type loci on mating proficiency

Strain[a]	Genotype				Mating phenotype[b]	Mating efficiency[c]
	HML	*MAT*	*HMR*	*ARD1*		
T8-1C0K	α	**a**	**a**	+	**a**	[1.0], [1.0][d]
T109.6-3C	α	**a**	**a**	+	**a**	1.0
M126-4C	α	**a**	**a**	+	**a**	0.63
M126-9D	α	**a**	**a**	+	**a**	1.0
T8-1D0K	α	α	**a**	+	α	1.1, 0.74
T109.6-3A	α	α	**a**	+	α	1.12
T8-1A0K	α	**a**	**a**	−	—[e]	2.1×10^{-5}, 8.2×10^{-3}
T109.6-2B	α	**a**	**a**	−	—	1.0×10^{-3}
M126-4D	α	**a**	**a**	−	—	4×10^{-4}
M126-5B	α	**a**	**a**	−	—	4×10^{-2}
T8-1B0K	α	α	**a**	−	α	0.84, 0.83
T109.6-4D	α	α	**a**	−	α	1.26
M126-1B	Δ	**a**	**a**	−	**a**	1.03
M126-2B	Δ	**a**	**a**	−	**a**	0.76
T8-1C2K	α	Δ	**a**	+	**a**	0.86, 1.40
T8-1A4k	α	Δ	**a**	−	**a** > α	0.30, 0.33 (as **a**); 0.07, 0.12 (as α)
MW2B	**a**	**a**	α	+	**a**	1.0, 1.0, 1.0
MW2A	**a**	**a**	α	−	**a**	1.16, 0.16, 0.39
MW2D	**a**	α	α	+	α	1.24, 1.16, 0.99
MW2C	**a**	α	α	−	—	0.01, 0.02, 0.14

[a] The indicated derivatives of strains T8-1A, T8-1B, T8-1C, and T8-1D (58) were generated as described in Table 1 and in Materials and Methods. Strains T109.6-2B, T109.6-3A, T109.6-3C, and T109.6-4D were generated by transformation of a diploid (DM109) with the Δ*ard*::*URA3* construction, followed by sporulation, as described in Materials and Methods. Strains M126-1B, M126-2B, M126-4C, M126-4D, M126-5B, and M126-9D were generated by sporulation of a diploid strain (DM126). Strain M147-3C was made diploid by transformation with an *HO*-containing plasmid, transformed with the *ard1*::*HIS3* construction, and sporulated to yield strains MW2A, MW2B, MW2C, and MW2D (in which the silent mating type loci are in inverse orientation), all as described in Materials and Methods.

[b] Mating phenotype was determined by patch mating on plates with DC14 and DC17 as the tester lawns.

[c] Quantitative matings were performed as described in Materials and Methods. Numbers given represent separate and independent determinations.

[d] All results normalized to the mating efficiency of this strain.

[e] —, Nonmater.

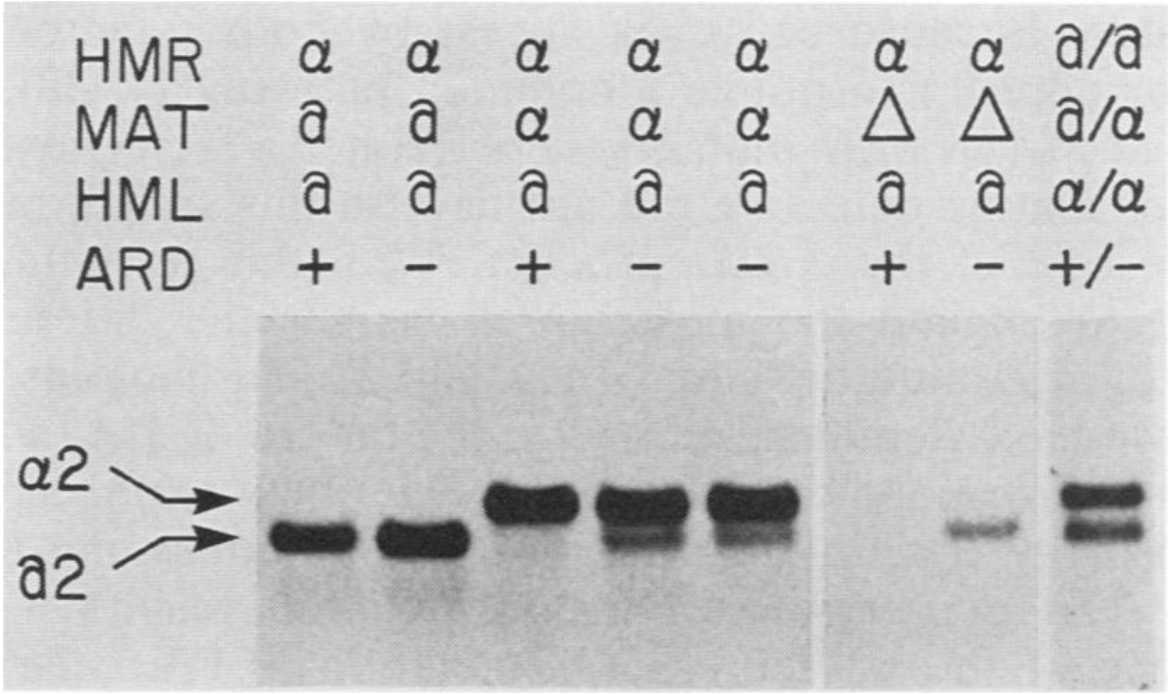

FIG. 3. Effect of an *ard1* mutation and inversion of the cryptic mating type loci on transcription of mating type genes. Poly(A)$^+$ RNA was prepared from the following strains (with the relevant genetic constitution indicated): (left to right) MW2D, MW2A, MW2B, MW2C, MW2COK, MW2B1K, MW2C1K, and YNNT8, subjected to electrophoresis, blotted, and hybridized to the same probe as used in Fig. 2.

TABLE 5. Suppression of the mating deficiency of *matα* mutations in *ard1* mutants

Strain[a]	Genotype		Mating efficiency[b]
	MAT	*ARD1*	
23α	α	+	[1.0][c]
T23	α	−	1.4
23α182	*matα2*	+	8.7×10^{-6}
T182	*matα2*	−	4.0×10^{-2}
23α113	*matα1*	+	1.4×10^{-5}
T113	*matα1*	−	4.2×10^{-3}

[a] The *ard1* derivatives were generated by transformation of the parental strains (Table 1) with the *ard1*::*URA3* construction, as described in Materials and Methods.

[b] Quantitative matings were performed as described in Materials and Methods.

[c] All results normalized to the mating efficiency of this strain.