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The *ARD1* Gene of Yeast Functions in the Switch between the Mitotic Cell Cycle and Alternative Developmental Pathways

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Summary

Mutations in the yeast gene *ARD1* lead to inability to respond to α-factor, inability to enter stationary phase, and inability to sporulate, suggesting an important role for the *ARD1* gene product in controlling the switch between the mitotic cell cycle and alternative cell fates. *MAT*a, *ard1* cells seem to be defective in the expression of all a-specific functions, whereas *MAT*α, *ard1* cells respond normally to a-factor. We propose that *ARD1* is required for the expression of genes involved in a-mating functions, stationary phase, and sporulation. The *ARD1* gene has been cloned and sequenced; there is weak homology between the C terminus of the *ARD1* protein, the C-terminal region of *MAT*α2, and the homeo box.

Experimental Procedures

Strains

Yeast strains used are listed in Table 1.

Media

Yeast strains were grown in YPD or SC (Sherman et al., 1979). Synthetic complete deficient in sulfur was made by replacing the ammonium sulfate with equimolar ammonium chloride. Bacterial strain MB1000 was grown on LB + 100 μg/ml ampicillin (Maniatis et al., 1982). M13 clones were selected in strain JM101 and maintained on YT soft agar overlays containing X-gal and IPTG (Messing et al., 1981).

Yeast Genetics

Standard techniques were used for diploid construction, sporulation, and tetrad dissection (Sherman et al., 1979). Auxotrophic and drug resistance markers were scored on appropriate drop-out or drug supplemented plates (Sherman et al., 1979). Mutants resistant to α-factor were isolated and scored on low pH plates (Hartwell, 1980) on which was spread 10–20 μg of synthetic α-factor (Sigma) dissolved in 90% methanol. Cells were spread at a density of 10^6 cells/ml or less to reduce background growth due to barrier mediated destruction of α-factor. The *ard*1 marker was also scored by crossing unknown strains to testers carrying the *ard*1 mutation and monitoring sporulation in the diploid.

Phenotype Tests

Bud ratios were determined by fixing cells in formaldehyde (Pringle and Mor, 1975), vortexing in the presence of glass beads to disrupt cell clumps, and counting budded and unbudded cells with a hemocytometer. More than 300 cells were counted for each determination. Quantitative matings were done by mixing various ratios of log phase cells with testers A23**a** or A24α, filtering them onto nitrocellulose discs, and incubating them on YPD plates for 4 hr. Cells were washed from the plates, vortexed to disrupt clumps, and suitable dilutions were spread on YPD plates to determine viable cells and on selective plates to determine diploid formation. This test also served as a test for agglutination; cells that had agglutinated required vigorous vortexing to disrupt the mating mixture, while nonagglutinated cells dispersed readily. Heat shock assays were performed by diluting stationary phase cells to 1×10^6/ml in water and treating them at 54°C for 5 min, then plating serial dilutions on YPD plates. Qualitative glycogen measurements were made by inverting YPD plates containing 4-day-old cultures of cells over iodine crystals in a closed container. After 3 min the plates were removed and the color of the colonies was noted. Barrier activity (Hicks and Herskowitz, 1976) was measured using *sst1* strain XMB4-12b as the test strain, and **a**-factor (Manney 1983) was measured using *sst2* strain RC757 as the tester.

DNA Manipulations

Bacterial plasmid or phage RF DNA was purified by alkali/SDS lysis, with the lysozyme and phenol steps omitted (Maniatis et al., 1982). Single-strand DNA for M13 sequencing was prepared as described (Schreier and Cortese, 1979). Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories or New England Biolabs and used in accordance with suppliers recommendations. Nick translations, Southern blotting, and hybridizations were done as described (Orr-Weaver et al., 1981). DNA purification from low melt agarose was achieved by the protocol of Dunn et al. (1984). Transformations of competent E. coli or of yeast spheroplasts involved standard techniques (Murray and Szostak, 1984).

DNA Sequencing

Dideoxy sequencing was performed on DNA fragments cloned in mp10 or mp11, using a commercial kit (New England Biolabs). Either a standard or a custom made primer was extended with Klenow polymerase in the presence of ^{32}P-dATP(800 Ci/mmol, Amersham). Gels were dried and autoradiographed without screens.

Table 1. Strains

DM11	leu2 his3 trp1 ura3 + + + leu2 + + + cyh2 can1 ard1-1
DM26	ade1 trp1 ard1-1 ade1 + +
DM40	ade1 trp1 ard1-1 + + + + + + + + + + + + ade2 trp5 his5 leu1 pet1 arg4 thr1 lys1 met1
DM47	spo13 leu2 ura3 ade2 lys1 lys2 his6 aro7 + + + + + + + + + + + + his3 trp1 can1 ard1-1
DM19-37C	leu2 ura3 his3 ard1-1 + + leu2 ura3 + ard1-1 trp1 cyh2
DM63	his3 leu2 can1 ard1::HIS3 + + + + + his3 + + + lys1 trp1 ura3 arg4 tcm1
DM64	his3 trp1 ura3 arg4 tcm1 + + + his3 + + + + can1 leu2 ard1::HIS3
DM100	ura3 leu2 his3 + ard1::HIS3 + + ura3 + his3 trp1 ard1::HIS3 can1 tcm1
TA405	leu2 his3 can1 leu2 his3 can1
TA405-1A	leu2 his3 can1 **a**
T8-1A	leu2 his3 can1 ard1::HIS3 **a**
T8-1B	leu2 his3 can1 ard1::HIS3 alpha
T8-1C	leu2 his3 can1 **a**
T8-1D	leu2 his3 can1 alpha
A23	thr4 **a**
A24	thr4 alpha
M43-1C	ura3 arg4 ade2 alpha
M91-1C	his6 sst2 can1 ard1::HIS3 alpha
M91-19D	his6 met1 sst2 can1 ard1::HIS3 **a**
RC757	his6 met1 sst2 can1 cyh2 alpha
XMB4-12b	sst1 ilv3 arg9 ura1 **a**

Table 2. Segregation of *ard*1 Relative to Other Markers

Diploid	Markers	PD	NPD	TT
DM11	ura3 ard1	8	6	11
DM11	trp1 ard1	10	15	9*
DM26	trp1 ard1	13	11	15*
DM40	arg4 ard1	52	0	1†
DM63	his3 arg4	12	0	0

* The pooled *ard1 trp1* values were used to calculate an *ard1* cen8 distance of 16.5 cM by the formula 1/2 SDS × 100 (Mortimer and Schild, 1980)

† The *ard1 arg4* distance of 0.9 cM was calculated by the formula TT + 6NPD/2(PD + NPD + TT) × 100 (Mortimer and Schild, 1980)

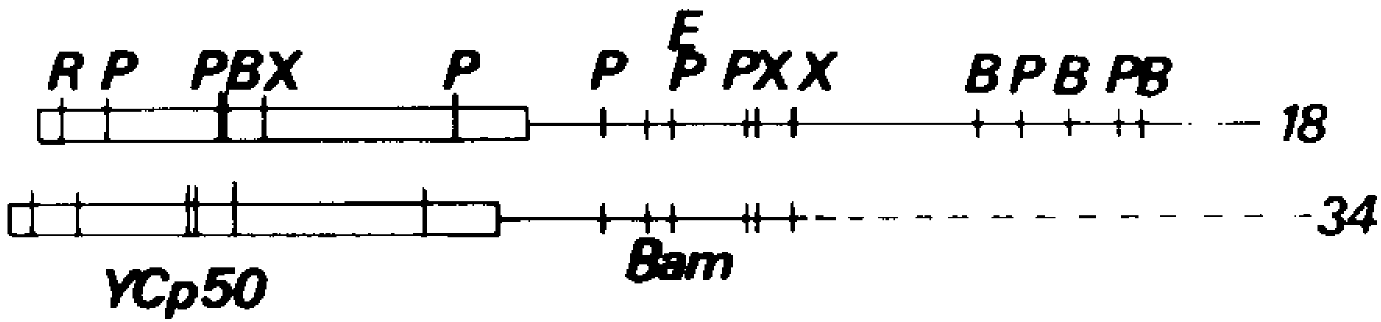

Figure 1. Restriction Maps of Plasmids YCp(ARD)18 and YCp(ARD)34

The sites of cleavage for enzymes Bgl II (B), Eco RI (E), Pst I (P), Xho I (X), and Bam HI (Bam) are shown. The region where clone 34 diverges from clone 18 is shown as a dashed line. The *ARD1* gene was found on clone 18 between the leftmost end of the insert and the Eco RI site of the insert; the *ARG4* gene was found between rightmost Bgl II site and the rightmost end of the insert. *ARD1* and *ARG4* are separated by 8 kb.

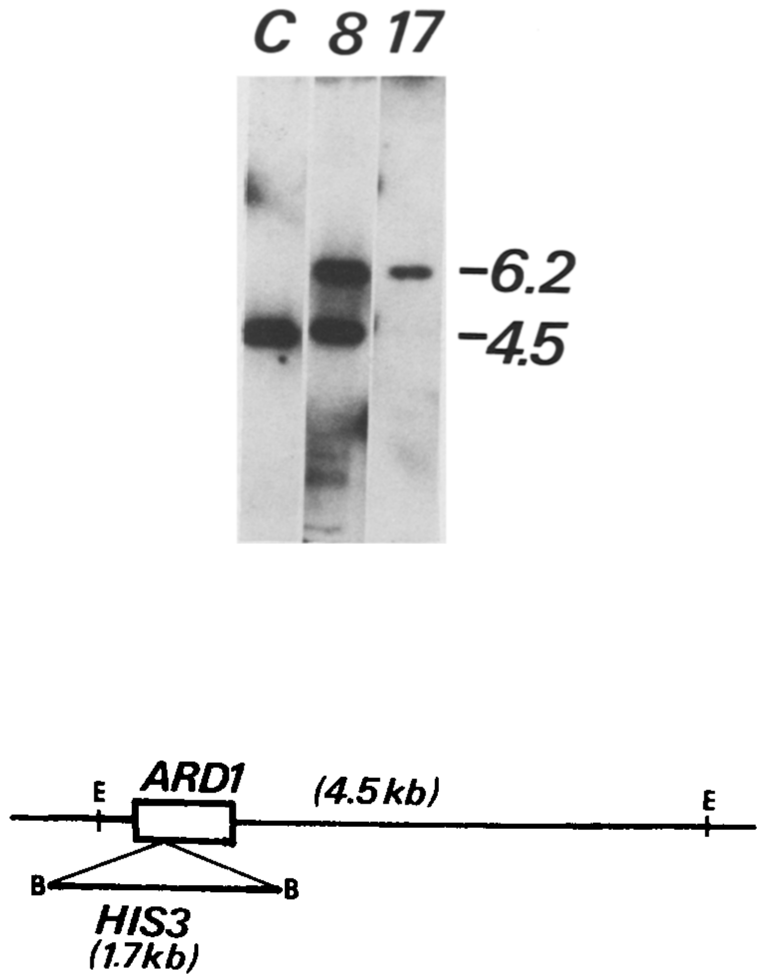

Figure 2. Southern Blot of *ARD1* and Disruption Strains

Total DNA was isolated from strain TA405 (lane C), T8 (lane 8), and T17 (lane 17), digested with Eco RI, blotted to nitrocellulose, and hybridized with an *ARD1* probe. The wild-type *ARD1* band is 4.5 kb; this is increased to 6.2 kb by the *HIS3* insertion. The line drawing shows the *ARD1* coding sequence as an open box, and shows the positions of the Eco RI (E) and Bam HI (B) sites within the *ARD1* region.

Table 3. Effect of *ARD1* on Entry into Stationary Phase

Strain	5 min Heat Shock % Survival*			Stationary % Survival†	% Budded Cells	Glycogen Accumulation
	YPD	SC	SC-S	SC		
T8-1A (ard1)	0.9	0.010	0.025	15	40	–
T8-1B (ard1)	1.3	0.007	0.006	11	43	–
T8-1C (ARD1)	89	3.0	8.3	66	9	+
T8-1D (ARD1)	110	5.0	3.9	80	15	+

* Strains were grown for 3 days in the designated medium, diluted to 10^6 cells/ml, then shocked at 54°C prior to plating on YPD plates.
† Strains were maintained in SC medium at 30°C, and the viability of the cells after 4 days was measured relative to the number of viable cells after overnight growth.

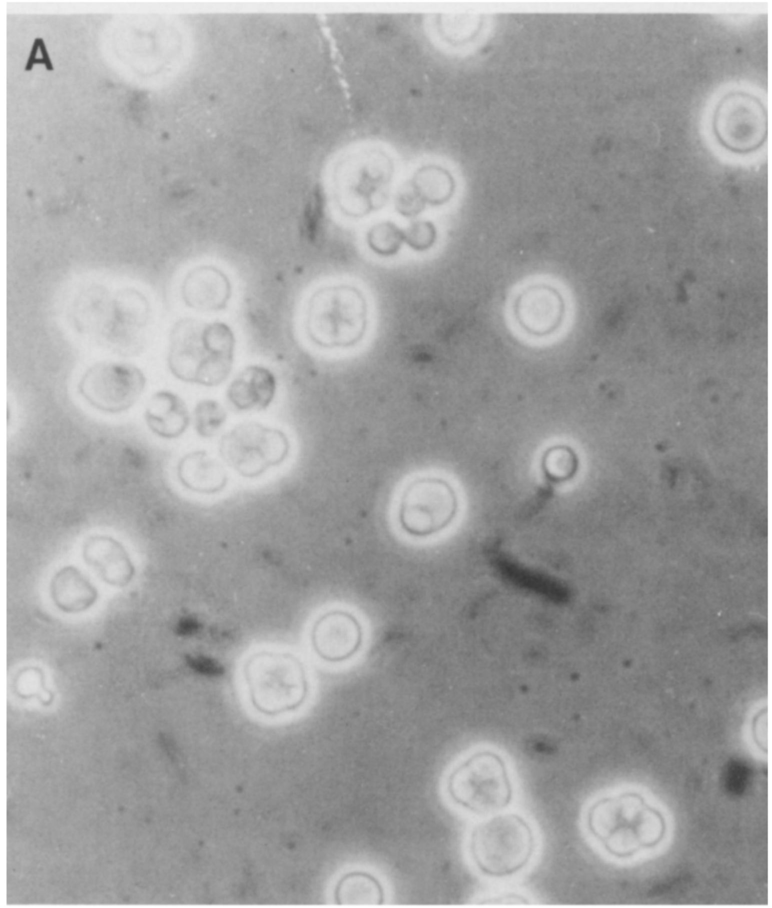

Figure 3. Sporulation in *ARD1* and *ard1::HIS3* Strains

Strains TA405 (A), T8 (B), and T17 (C), were maintained on sporulation plates for 6 days, then monitored for asci. The *ARD*+ homozygote TA405, and the heterozygote T8 showed asci, but none were found in the homozygous disruption strain T17.

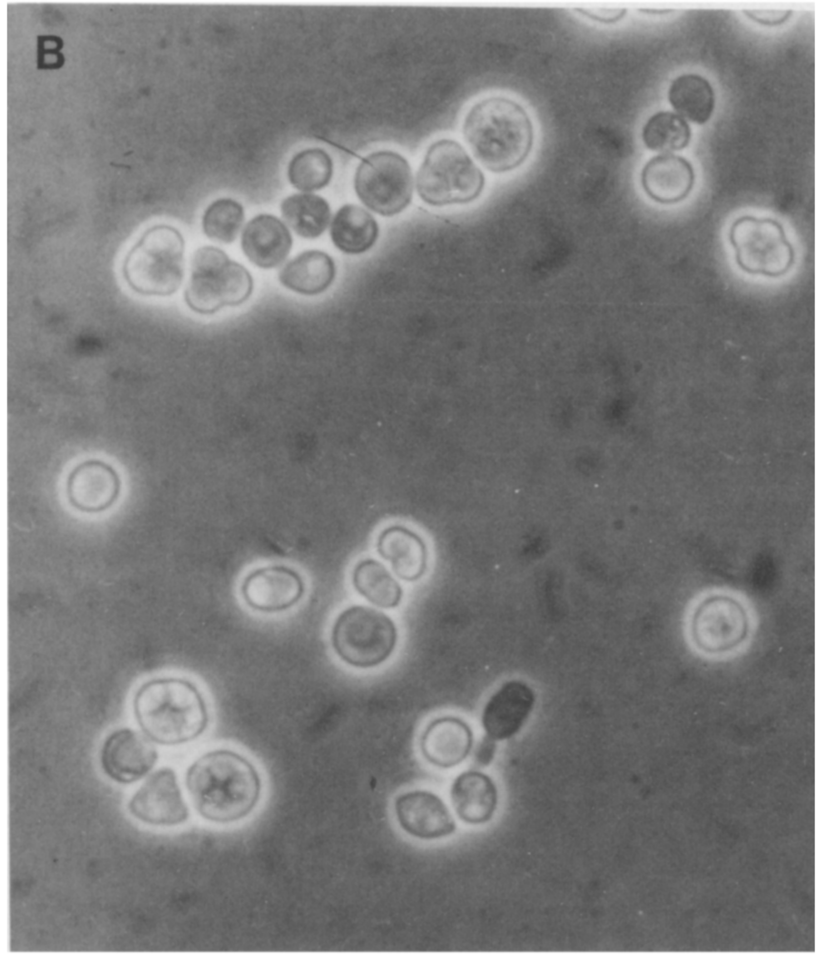

Figure 3. Sporulation in *ARD1* and *ard1::HIS3* Strains

Strains TA405 (A), T8 (B), and T17 (C), were maintained on sporulation plates for 6 days, then monitored for asci. The *ARD*[+] homozygote TA405, and the heterozygote T8 showed asci, but none were found in the homozygous disruption strain T17.

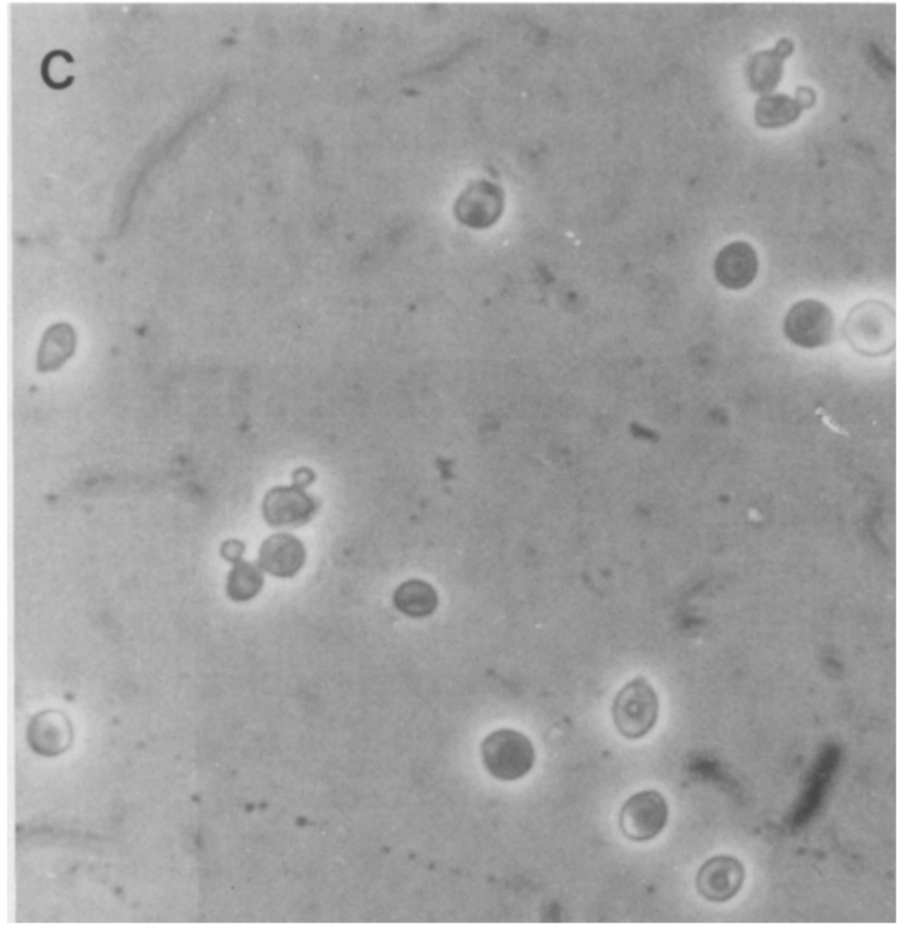

Figure 3. Sporulation in *ARD1* and *ard1::HIS3* Strains

Strains TA405 (A), T8 (B), and T17 (C), were maintained on sporulation plates for 6 days, then monitored for asci. The *ARD*$^{+}$ homozygote TA405, and the heterozygote T8 showed asci, but none were found in the homozygous disruption strain T17.

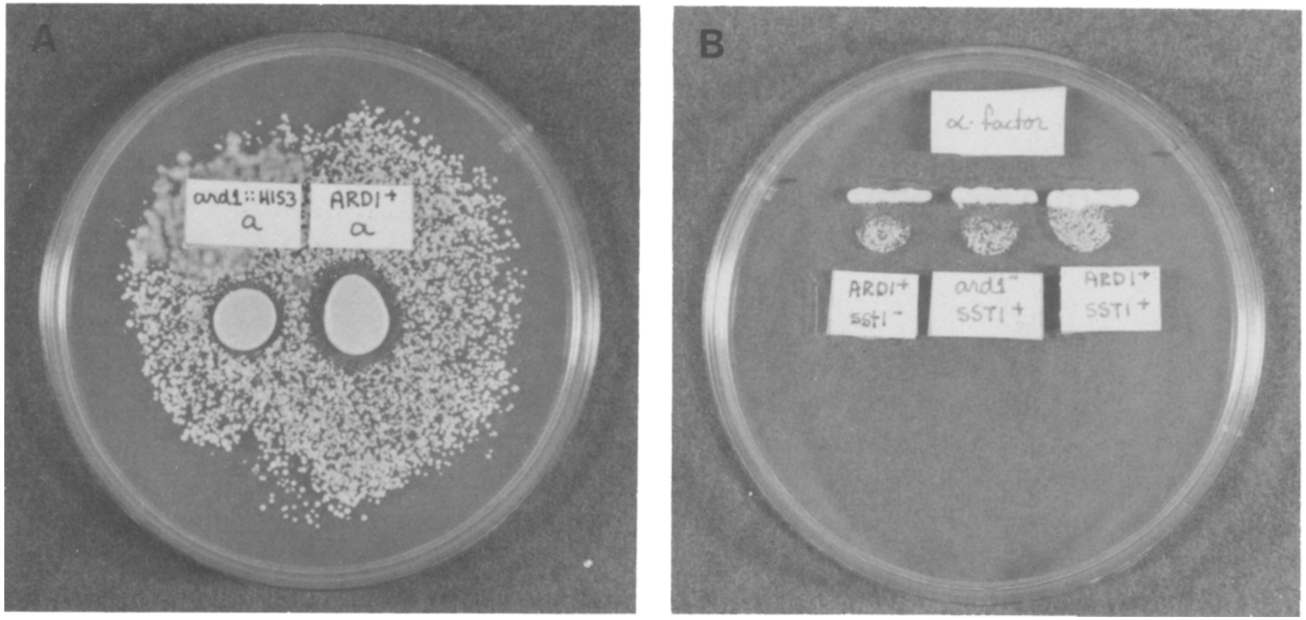

Figure 4. **a**-Specific Gene Expression

(A) Production of **a**-Factor in *ARD1* and *ard1::HIS3* Strains. Spots of 10 μl of exponentially growing T8-1A (**a**, *ard1*) and T8-1C (**a**, *ARD1*$^+$) at 1 × 10^7 cells/ml were placed on a lawn of s strain RC757, which is supersensitive to the affects of **a**-factor. The sizes of the halos of inhibition are a measure of **a**-factor production of the two strains.

(B) Barrier activity in *ARD1* and *ard1::HIS3* strains. Streaks of cells from strains XMB4-12b (**a**, sst1, ARD1$^+$), T8-1A (**a**, SST1$^+$, ard1), and T8-1C (**a**, SST1$^+$, ARD1$^+$), were interposed between a streak of purified α-factor and the barrier defective strain XMB4-12b. Both strains T8-1A and XMB4-12b failed to prevent inhibition of growth of the tester strain by the diffusing α-factor.

-260 -240 -220
GAATTCTAAGTATAATCTCCATGAGACTTCCAGGACAAATATTTTCTCTTAAAGGGGCT

-200 -180 -160
TGCTTTAGTAGTATTGCATCCAATGCTAATTTGTCTTTATTGATCTCTAGGCTCTATCCT

-140 -120 -100
CACTTTCTCGCGTCAATTGGTTTTTTGAACAGAAAAAAAAAGCACCGTCTGAAAAGTTTG

-80 -60 -40
AAACCTTTTGTCAGCAACGAGTAATTGCCAAGTGAAAAGGGAAACCTAAATACATACGAT

-20 1 21
CAAGCTCCAAAATAAACTTCGTCAACCATGCCTATTAATATTCGCAGAGCGACAATCAAT
MetProIleAsnIleArgArgAlaThrIleAsn

41 61 81
GACATTATATGTATGCAAAATGCCAACCTGCATAACCTACCCGAAAATTATATGATGAAA
AspIleIleCysMetGlnAsnAlaAsnLeuHisAsnLeuProGluAsnTyrMetMetLys

101 121 141
TATTATATGTATCATACTCTCTCGTGGCCAGAGGCTTCGTTTGTTGCTACTACCACCACT
TyrTyrMetTyrHisThrLeuSerTrpProGluAlaSerPheValAlaThrThrThrThr

161 181 201
TTGGACTGTGAAGATAGTGATGAACAAGATGAGAACGACAAATTGGAATTGACCTTAGAT
LeuAspCysGluAspSerAspGluGlnAspGluAsnAspLysLeuGluLeuThrLeuAsp

221 241 261
GGGACCAATGACGGCAGGACAATCAAGTTGGATCCAACATACTTGGCTCCGGGCGAAAAA
GlyThrAsnAspGlyArgThrIleLysLeuAspProThrTyrLeuAlaProGlyGluLys

281 301 321
TTAGTTGGATACGTTCTCGTGAAGATGAACGACGACCCCGACCAGCAAAACGAGCCACCA
LeuValGlyTyrValLeuValLysMetAsnAspAspProAspGlnGlnAsnGluProPro

341 361 381
AATGGTCATATTACTTCCTTAAGTGTCATGAGAACCTATAGAAGGATGGGGATTGCCGAA
AsnGlyHisIleThrSerLeuSerValMetArgThrTyrArgArgMetGlyIleAlaGlu

401 421 441
AACTTAATGAGACAAGCTTTATTTGCGTTGAGAGAAGTTCATCAAGCCGAATATGTTTCC
AsnLeuMetArgGlnAlaLeuPheAlaLeuArgGluValHisGlnAlaGluTyrValSer

461 481 501
TTGCATGTGAGACAATCTAATAGAGCAGCTTTACACTTGTACAGGGACACATTGGCGTTT
LeuHisValArgGlnSerAsnArgAlaAlaLeuHisLeuTyrArgAspThrLeuAlaPhe

521 541 561
GAGGTGTTGAGTATCGAGAAAAGCTACTATCAGGATGGTGAAGATGCATACGCCATGAAG
GluValLeuSerIleGluLysSerTyrTyrGlnAspGlyGluAspAlaTyrAlaMetLys

581 601 621
AAGGTTTTAAAATTAGAGGAGTTGCAGATAAGCAACTTCACCCATCGCCGTTTGAAAGAG
LysValLeuLysLeuGluGluLeuGlnIleSerAsnPheThrHisArgArgLeuLysGlu

641 661 681
AATGAGGAAAAACTAGAAGACGATCTAGAAAGCGATCTACTAGAGGATATCATTAAGCAA
AsnGluGluLysLeuGluAspAspLeuGluSerAspLeuLeuGluAspIleIleLysGln

701 721 741
GGCGTAAATGATATCATTGTATAATTAAATCAACCTATATAAACGTAGTATATTTTCATC
GlyValAsnAspIleIleVal

761 781 801
CAGGCTTCTTCACAAGCTCTGTTAGCTCTTTTTGAGCACGGTTCTAAACACTGCAGTGCT

821 841 861
AATTCGGTAGAATTGTGTGAGCATGAATAGTAAGGAAACTGTGCATGCAAGCTTTCTTTT

881 901 921
TTTATATATGTAATATCCGGCAGATAGGATATCTTAATTACATCTATCTCGTCTGCAAGA

941 961 981
AAAAGTTCACGAATGCATCTAATGTTTAGACATCATAGAAATGCATAAAAATGAAAATGG

1001 1021
CTACCCTATTCCTTTTCATAAACCACTTTATCGA

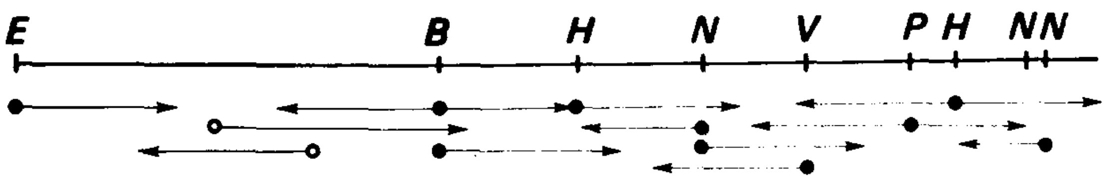

Figure 5. Sequence of the *ARD1* Gene and Surrounding Region

The sequencing strategy is diagrammed below the sequence. The arrows with open circles are sequences derived using custom primers; those with closed circles were obtained using the standard primer. All of the coding sequence was determined from both strands or from several independent gels covering the same region. The A of the most likely AUG has been designated position +1, the ORF from this codon extends for 238 amino acids to a TAA termination codon.

ARD	Tyr	Gln	Asp	Gly	Glu	Asp	Ala	Tyr	Ala	Met	Lys	Lys	Val	Leu	Lys	Leu	Glu
MAT	Tyr	Leu	Asp	Thr	Lys	Gly	Leu	Glu	Asn	Leu	Met	Lys	Asn	Thr	Ser	Leu	Ser
UBX	Tyr	Leu	Thr	Arg	Arg	Arg	Arg	Ile	Glu	Met	Ala	His	Ala	Leu	Cys	Leu	Thr
ARD	Glu	Leu	Gln	Ile	Ser	Asn		Phe	Thr	His	Arg	Arg	Leu	Lys	Glu	Asn	Glu
MAT	Arg	Ile	Gln	Ile	Lys	Asn	Trp	Val	Ser	Asn	Arg	Arg	Arg	Lys	Glu	Lys	Thr
UBX	Glu	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met	Lys	Leu	Lys	Lys
ARD	Glu	Lys	Leu	Glu	Asp	Asp	Leu	Glu	Ser	Asp	Leu	Leu	Glu	Asp	Ile	Ile	
MAT	Ile	Thr	Ile	Ala	Pro	Glu	Leu	Ala	Asp	Leu	Leu	Ser	Gly	Glu	Pro	Leu	
UBX	Glu	Ile	Gln	Ala	Ile	Lys	Glu	Leu	Asn								

Figure 6. Comparison of the C-Terminal Regions of *ARD1*, *MAT* 2, and the *UBX* Homeo Box

Identical amino acids are shaded. The *ARD1* peptide sequence shown starts at residue 181, that of *MAT* 2 starts at residue 156, and that of the homeo box starts at residue 25 (Shepherd et al., 1984).