

# Identification and characterization of genes and mutants for an *N*-terminal acetyltransferase from yeast

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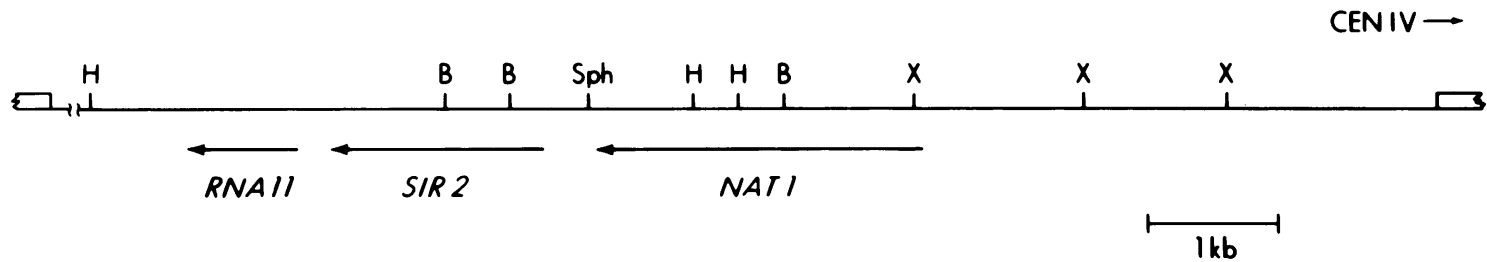
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A gene from *Saccharomyces cerevisiae* has been mapped, cloned, sequenced and shown to encode a catalytic subunit of an N-terminal acetyltransferase. Regions of this gene, *NAT1*, and the chloramphenicol acetyltransferase genes of bacteria have limited but significant homology. A *nat1* null mutant is viable but exhibits a variety of phenotypes, including reduced acetyltransferase activity, derepression of a silent mating type locus (*HML*) and failure to enter  $G_0$ . All these phenotypes are identical to those of a previously characterized mutant, *ard1*. *NAT1* and *ARD1* are distinct genes that encode proteins with no obvious similarity. Concomitant overexpression of both *NAT1* and *ARD1* in yeast causes a 20-fold increase in acetyltransferase activity *in vitro*, whereas overexpression of either *NAT1* or *ARD1* alone does not raise activity over basal levels. A functional iso-1-cytochrome *c* protein, which is N-terminally acetylated in a *NAT1* strain, is not acetylated in an isogenic *nat1* mutant. At least 20 other yeast proteins, including histone H2B, are not N-terminally acetylated in either *nat1* or *ard1* mutants. These results suggest that *NAT1* and *ARD1* proteins function together to catalyze the N-terminal acetylation of a subset of yeast proteins. **Key words:** *Saccharomyces cerevisiae*/N-terminal acetyltransferase/gene/mutant

**Table I.** Acetyltransferase activity in NAT1 and *nat1-1* strains from two tetrads

Tetrad	Activity (c.p.m.) <sup>a</sup>		<i>NAT1</i>	<i>TRP1</i>
	25°C	37°C		
2a	3850	1100	+	-
2b	3200	350	-	+
2c	3450	500	-	+
2d	3700	1100	+	-
3a	3300	950	+	-
3b	3450	1100	+	-
3c	3800	350	-	+
3d	3000	400	-	+

<sup>a</sup>Acetyltransferase assays were performed as described in Materials and methods. Assays at 37°C were with extracts that had been pre-heated at 45°C for 8 min, while the assays at 25°C were with unheated extracts.



**Fig. 1.** Restriction map of pJM100. pJM100 contains ~15 kb of genomic DNA, including three genes: *NAT1*, *SIR2* and *RNA11*. The map order of these three genes with respect to the centromere (*CENIV*) is indicated. The map order was determined by tetrad analysis from crosses of appropriately marked haploid strains (see Materials and methods). Restriction sites are abbreviated as follows: *Bam*HI (B), *Xho*I (X), *Hind*III (H), *Sph*I (Sph).

NATI: 1 4 8 R K K Y W E A F L G Y R A N W T S L A V A Q D V 1 7 1

\* \* \* \* \*

CAT: 1 8 R K E H F E A F Q S V A Q C T Y N Q T V Q L D I 4 1

NATI: 6 8 1 G E K L I E T S - T P M E D - F A T E F Y N N Y S - - M Q V R E D E R D Y 7 1 3

\* \* \* \* \*

CAT: 8 0 G E L V I W D S V H P C Y T V F H E Q T E T F S S L W S E Y H D D F R Q F 1 1 6

10 20 30 40 50 60 70  
 TCAATGAGAGGAGCTCTGAGAAAGCTTGGATTGATATATATACGCTATTATCTTTAAGGGAATACGC

80 90 100 110 120 130 140 150  
 TAATTTCTATTATTGTACACCTGGAGACATGATAGCCGTGAATGTGCCACCAATTCGATCACTTCTGTAATGAG

160 170 180 190 200 210 220  
 CTCGCTGGTTCGCTTGCACCTTTTACCTCGCTCGCTCGAAACAGAACATAATACAGACTAAGAGGGAAGAG

230 240 250 260 270 280 290 300  
 CGAAATGAAAAGTCTTACACTCAAAAGTCAAACTTTTAAAGAGTGTTCAGATTCTGTTTAAAGACA

310 320 330 340 350 360 370  
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 MetSerArgLysArgSerThrLysProLysProAlaAla

380 390 400 410 420 430 440 450  
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460 470 480 490 500 510 520  
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530 540 550 560 570 580 590 600  
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 TyrTyrSerValGlyGlyLysAspAlaAlaSerTyrValAlaAanAlaIleArgIleGluAlaAlaSer

610 620 630 640 650 660 670  
 GCATCCCAATCTCGCTCATGTATTAGCTATCTCATGAAACCCAAAGACTACAAAGAACTATTAAATGG  
 AlaSerProIleCysCysHisValLeuGlyIleTyrMetArgAsnThrLysGluTyrLysGluSerIleLysTyr

680 690 700 710 720 730 740 750  
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760 770 780 790 800 810 820  
 GCGCATTTCAAAAATCTTGTAGCTCCAGGAAAAATTTGGGAAGCATCTCTGCTTACCGTGCCAACTGGACA  
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830 840 850 860 870 880 890 900  
 TCAATGGCTGTGGCAAGATGCAAGCGGTGAGAGCAACGACTTAACTGCTTATCTCAAGTGTGAAAACCT  
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910 920 930 940 950 960 970  
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980 990 1000 1010 1020 1030 1040 1050  
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1060 1070 1080 1090 1100 1110 1120  
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1280 1290 1300 1310 1320 1330 1340 1350  
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1360 1370 1380 1390 1400 1410 1420  
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1430 1440 1450 1460 1470 1480 1490 1500  
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1510 1520 1530 1540 1550 1560 1570  
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1730 1740 1750 1760 1770 1780 1790 1800  
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1810 1820 1830 1840 1850 1860 1870  
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1880 1890 1900 1910 1920 1930 1940 1950  
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1960 1970 1980 1990 2000 2010 2020  
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2030 2040 2050 2060 2070 2080 2090 2100  
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2630 2640 2650 2660 2670 2680 2690 2700  
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2710 2720 2730 2740 2750 2760 2770  
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2780 2790 2800 2810 2820 2830 2840 2850  
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2860 2870 2880 2890 2900 2910 2920  
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3010 3020 3030 3040 3050 3060 3070  
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3080 3090 3100 3110 3120 3130 3140 3150  
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3160 3170 3180 3190 3200 3210 3220  
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3230 3240 3250 3260 3270 3280 3290 3300  
 TGAAGAGATGAAGCCACTTCCACCTATTTCAAGAAATAGGCATCGCTCGGTAGACACATTCAACCAATTT

3310 3320 3340 3350  
 TTCCCTCATCGGCACATTAAGCTGGATGACCATCCACATATG

SIR 2

Fig. 2. DNA sequence of *NAT1*. The sequence of *NAT1* from nucleotide 1 to nucleotide 2975 was determined by standard methods (see Materials and methods). The sequence from nucleotides 2976 to 3354 is from Shore *et al.* (1984). The beginning of the *SIR2* ORF is indicated by an arrow. There is complete agreement in the overlap region from nucleotides 2710 to 2975, sequenced by both our group and Shore *et al.* (1984). The putative protein sequence of *NAT1* is indicated.

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**Table II.** Effect of *nat1* mutation on survival to heat shock and mating efficiency

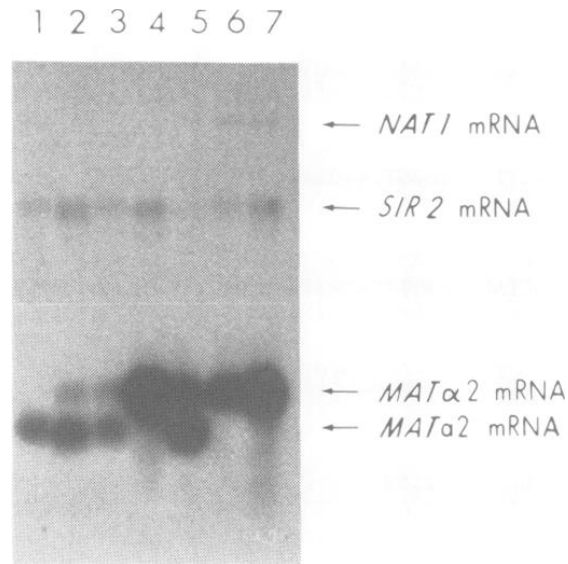
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Strain	Relevant genotype	% Survival after heat shock <sup>a</sup>	Relative mating efficiency <sup>b</sup>
W303-1a	<i>NAT1</i>	91	1.0
AMR1	<i>nat1</i>	18	$6.6 \times 10^{-3}$

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<sup>a</sup>Strains were grown in YPD at 30°C for 4 days and then subjected to a 5-min heat shock at 54°C as described in Materials and methods.

<sup>b</sup>Strains were grown to mid-log phase in YPD at 30°C. Dilutions were made and aliquots were plated onto either YPD plates (to determine the number of viable cells) or onto SD plates spread with a thin lawn of the MAT $\alpha$  strain 217 (to determine mating ability). The mating efficiency is the number of cells that mated per total number of cells; it was arbitrarily defined as 1.0 for W303-1a.



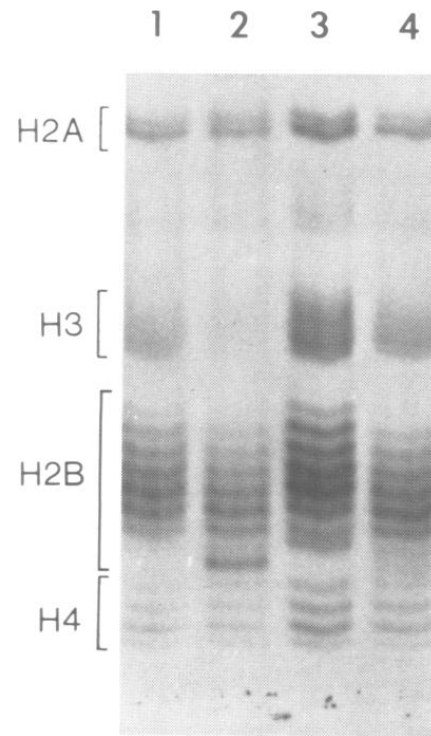
**Fig. 3.** Derepression of *HML* in *nat1* and *ard1* single mutants and in *nat1 ard1* double mutant strains. Total RNA was probed for  $\alpha 2$  and  $a 2$  transcripts, using  $^{32}\text{P}$ -labeled anti-sense RNA. **Lanes 1 and 2**, *MATa NAT1* (W303-1a) and *MATa nat1* (AMR1), respectively, — an isogenic set; **lanes 3 and 4**, *MATa nat1 ard1* and *MAT $\alpha$  nat1 ard1* double mutants, respectively; **lane 5**, *MATa sir1 sir3* strain (RS19); **lanes 6 and 7**, *MAT $\alpha$  ARD1* (9-3A) and *MAT $\alpha$  ard1* (9-4D), respectively, — an isogenic set. Note the darker  $\alpha 2$  transcript band in the *MAT $\alpha$  ard1* strain (**lane 7**) and the *MAT $\alpha$  nat1 ard1* double mutant (**lane 4**). This is due to partial derepression of *HML* in addition to normal transcription from the *MAT* locus. This effect is also seen in *MAT $\alpha$  nat1* strain (data not shown). The same filter was probed with a  $^{32}\text{P}$ -labeled anti-sense *SIR2* probe as a control. A short region of this riboprobe also contained anti-sense *NAT1* RNA (see Materials and methods), thus also allowing visualization of the *NAT1* transcript.

**Table III.** Acetyltransferase activities of *nat1* and *ard1* single and double mutants

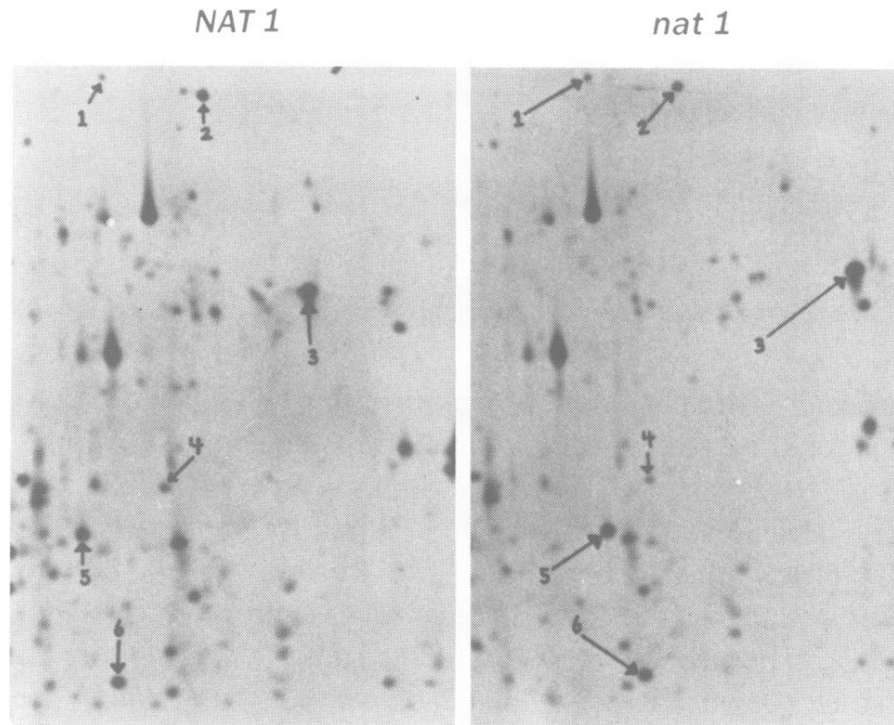
Strain	Relevant genotype	Acetyltransferase activity (c.p.m.) <sup>a</sup>	
		No pre-heating	8 min at 45°C
RS304-1a	<i>NAT1 ARD1</i>	12000	6050
RS304-1b	<i>NAT1 ard1</i>	10400	2400
RS304-1c	<i>nat1 ARD1</i>	8250	2500
RS304-1d	<i>nat1 ard1</i>	9200	2600

<sup>a</sup>Assays were for 20 min at 25°C as described in Materials and methods.





**Fig. 4.**  $^3\text{H}$ -Labeled histones from *nat1* and *ard1* single mutants and their isogenic parents. Yeast histones were labeled *in vivo* with [ $^3\text{H}$ ]acetate, isolated and separated on a 12% Triton-acid-urea gel, which was then processed for fluorography. **Lane 1**, strain W303-1a (*NAT1*); **lane 2**, AMR1 (*nat1*); **lane 3**, 9-3A (*ARD1*); **lane 4**, 9-4D (*ard1*). The histone H2B bands from the *nat1* mutant (**lane 2**) and the *ard1* mutant (**lane 4**) are shifted down by one 'step', a movement consistent with the loss of the N-terminal acetyl group. The very dark band low in the H2B group of the *nat1* mutant (**lane 2**) is not seen in the *ard1* mutant (**lane 4**). This difference in the pattern is probably due to the fact that the strains are not isogenic.



**Fig. 5.** Two-dimensional gels of [ $^{35}\text{S}$ ]methionine-labeled proteins from *NAT1* and *nat1* strains. *NAT1* (W303-1a) and *nat1* (AMR1) strains were briefly labeled with [ $^{35}\text{S}$ ]methionine, total protein was isolated and separated on two-dimensional gels, which were then processed for fluorography. The first dimension is an isoelectric focusing gel; the basic side is to the right. The second dimension is a standard denaturing gel with the direction of migration from top to bottom. The regions shown contain proteins of  $\sim 30\text{--}80$  kd, with pIs ranging from  $\sim 4$  to 8. Identical regions from the two gels are shown. Six prominent protein spots are indicated that shift toward the basic side in the *nat1* strain. The shifts are consistent with lack of acetylation of the N termini of these proteins in the *nat1* strain.

**Table IV.** Overproduction of acetyltransferase activity *in vitro*

Plasmids	Overproduced protein(s)	Specific activity (c.p.m./ $\mu$ g protein) <sup>a</sup>
pVT100-U + pVT103-L	—	1550
pVT100-U + pJM124	NAT1	1500
M33p5 + pVT103-L	ARD1	1150
M33p5 + pJM124	NAT1 + ARD1	33 700

<sup>a</sup>Extracts were prepared as described in Materials and methods, but were not subjected to pre-heating prior to assaying. Assays were at 25°C for 20 min. Extracts were diluted 1:10 and 1  $\mu$ l was used per assay. With undiluted extracts, the NAT1/ARD1 overproducing extract gave 8–10 times more activity than the other extracts.

## Materials and methods

### ***Yeast strains, media and strain manipulations***

Yeast were grown at 30°C in either YPD medium or in SD medium plus appropriate supplements (Sherman *et al.*, 1986). Cells were transformed by standard spheroplasting techniques (Sherman *et al.*, 1986). Diploid construction, sporulation, tetrad dissection and analysis were done by standard methods (Sherman *et al.*, 1986). The following strains were used: YS7p/4d: *MAT $\alpha$  ade2 ura3 leu2 trp1 his3*; MCSY314: *MAT $\alpha$  ade ura1-1 lys2-1 thr4 met4-1 can1-100 nat1-1*; W303-1a: *MAT $\alpha$  ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100*; AMR1: Isogenic to W303-1a plus *nat1-5 :: LEU2*; J24: *MAT $\alpha$  ade1 ade2 ura1 his7 lys2 tyr1 gall-1 rna1<sup>ts</sup>*; 9-3A: *MAT $\alpha$  ura3 leu2 his3*; 9-4D: isogenic to 9-3A plus *ard1 :: URA3* (Whiteway *et al.*, 1987); RS19: *MAT $\alpha$  sir1-1 sir3 :: LEU2 ade6 arg4 leu2 trp1 RME1*; DF1: *MAT $\alpha$  ade2 ura3-52 trp1-1 lys2 his3 his7 leu2-3,112 NAT1 :: LEU2*; DF2: *MAT $\alpha$  ade2-101 ura leu2-3,112 tyr1 his rna1<sup>ts</sup> lys2*; 216: *MAT $\alpha$  his1*; 217: *MAT $\alpha$  his1*; B-7470: *MAT $\alpha$  CYC1-793 cyc7-67 lys5-10 ura3-52*; B-7658: isogenic to B-7470 plus *nat1-3 :: URA3*.

### ***Null mutations and plasmids***

The *nat1-3 :: URA3* null mutation was constructed by deleting a *HindIII* fragment from the *NAT1* coding sequence (see Figure 1) and inserting a 1.1 kb *HindIII* fragment containing the *URA3* gene. The mutation was placed in the genome by gene transplacement (Rothstein, 1983). The construction of the *nat1-5 :: LEU2* null mutation is described in the Results. M33p5 (a gift from M. Whiteway) contains the *ARD1* gene cloned behind the constitutive ADH promoter in a 2  $\mu$ m based *URA3* vector (Vernet *et al.*, 1987). pJM124 contains a functional *NAT1* gene (coding for the first 11 amino acids of phage T7 gene 10 protein plus four amino acids from linker sequences, followed by amino acids 7–853 of *NAT1*) cloned behind the ADH promoter in a 2  $\mu$ m based *LEU2-d* plasmid (Vernet *et al.*, 1987).

### ***Extracts and acetyltransferase assays***

In the original screen for a histone acetyltransferase mutant, cells were grown in 20 ml of YPD to a density of  $1 \times 10^7$  cells/ml, pelleted, resuspended in 1 ml assay buffer (75 mM Tris pH 8.2, 150 mM NaCl, 0.1 mM EDTA, 2 mM  $\beta$ -mercaptoethanol), and vortexed with an equal volume of glass beads in 30s pulses alternated with 1 min on ice for a total vortexing time of 4 min. Disrupted cells were centrifuged at 12 000 *g* for 10 min and pellets were resuspended in 0.225 ml assay buffer. Aliquots containing 0.5 mg total protein (Lowry *et al.*, 1951) were used in assays (Travis *et al.*, 1984). After the original screening, NAT assays including those shown in Tables I, III and IV, were done in the following manner. Strains were grown in 50 ml of YPD to  $2 \times 10^8$  cells/ml or in 100 ml of minimal medium to  $1 \times 10^8$  cells/ml. Cells were pelleted, washed once with water, and made into spheroplasts using the protocol of Celniker and Campbell (1982). All manipulations from this point on were done at 4°C or on ice. Spheroplasts were transferred to Eppendorf tubes, washed with 1 ml of Buffer A (Celniker and Campbell, 1982) and pelleted. Nuclei were released by homogenization (30 strokes of a micro-pestle) in 0.1 ml Buffer A and pelleted in a microfuge. Nuclei were lysed by addition of 0.2 ml of NAT YLB [20 mM Tris pH 7.4, 10% glycerol, 1 M NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF]. After brief vortexing, the crude nuclear lysates were centrifuged for 10 min in a cold microfuge, and the supernatants used for assays. One  $\mu$ l of crude extract (containing between 3 and 7  $\mu$ g protein/ $\mu$ l) was used per 50  $\mu$ l assay. Assay conditions were those of Travis *et al.* (1984) except that extracts were placed at 45°C for 8 min just prior to assaying. Assays were at 25°C for 20 min. Reactions were stopped by the addition of 1.5 ml of cold 20% TCA. Precipitated proteins were collected on glass fiber filters (Schleicher and Schuell), washed twice with 5% TCA, once with methanol, dried and counted.

### **Mapping of *NAT1*, *SIR2* and *RNA11***

The linkage of *NAT1* and *TRP1* was established by crossing strain MCSY314 to YS7p/4d. The resulting tetrads were scored for the *nat1-1* mutation (by the *in vitro* acetyltransferase assay) and for tryptophan auxotrophy by replica plating. The linkage of *NAT1* and *RNA11* was established by crossing a *nat1-1* mutant strain to J24. The resulting haploid progeny were analyzed for both acetyltransferase activity *in vitro* and for temperature sensitivity.

The map order of the three genes *NAT1*, *SIR2* and *RNA11* with respect to the centromere of chromosome IV was determined by inserting the *LEU2*

gene into the *XhoI* site furthest upstream from *NAT1* (see Figure 1), and replacing wild-type DNA with this marked DNA by gene transplacement (Rothstein, 1983). This haploid strain, DF1 (constructed by D.Fossaceca), was then mated to an appropriately marked strain, DF2, creating a diploid which was heterozygous for the three markers: *rna11<sup>ts</sup>*, *trp1* and *LEU2* (marking the region upstream of *NAT1*). This diploid was sporulated and the resulting tetrads analyzed for crossovers between *LEU2* (*NAT1*) and *rna11<sup>ts</sup>*. Analysis of 157 tetrads resulted in five crossovers between the marked region upstream of *NAT1* and *rna11<sup>ts</sup>*. In all five cases, *LEU2* (*NAT1*) remained parental with respect to *TRP1*, while *RNA11* became tetratype with respect to both *TRP1* and *LEU2* (*NAT1*), indicating that the map order is *RNA11-SIR2-NAT1-CENIV-TRP1*, as shown in Figure 1.

### ***Sequencing of NAT1 and homology search***

Overlapping restriction fragments from the *NAT1* coding region were subcloned into either M13mp10 or M13mp11 (Messing and Vieira, 1982) permitting sequencing of both strands. Sequence analysis of the subclones was carried out by the dideoxy method of Sanger *et al.* (1977).

The *NAT1* sequence was compared to every sequence in the Protein Identification Resource databases (PIR, National Biomedical Research Foundation, 3900 Reservoir Road, N.W., Washington, DC 20007). These included PSQ (release 14.0, comprising 4721 sequences with a total of 1 118 494 residues) and New (release 31.0, comprising 1697 sequences with a total of 393 114 residues). Sequence alignments were performed using the BESTFIT program of the GCG sequence analysis package (University of Wisconsin Genetics Computer Group, U. of W. Biotechnology Center, 1710 University Ave., University of Wisconsin, Madison, WI, 53705). A variety of gap (G) and gap length (L) penalties were examined: G = 2.0, 5.0, and 7.5; L = 0.0, 0.05, 0.1, and 1.0. Sequence alignments between individual protein sequences, as opposed to searches of the PIR databases, were performed both with the entire sequence and with 50- or 100-residue segments of one of the sequences. Profile analysis was carried out by the method of Gribskov *et al.* (1987), using logarithmic weighting applied uniformly to each sequence used to generate the profile. Searches of the PIR databases were repeated three times with different values of gap and gap length weights: 3.0, 0.1 (G,L); 5.0, 0.05; and 10.0, 0.0.

### **Phenotype tests**

$\alpha$ -Factor sensitivity was determined by adding  $\alpha$ -factor (Sigma) to a final concentration of 5  $\mu\text{g/ml}$  to logarithmically growing yeast cultures in pH 5.0 YPD medium at a density of  $1-2 \times 10^7$  cells/ml. Three to four hours later, cells were examined microscopically for formation of mating structures. Quantitative matings were done by plating serial dilutions of logarithmically growing cells onto SD plates containing a lawn of either 216 or 217 mating type tester strains to determine diploid formation. Serial dilutions were also plated onto YPD plates to determine total cell number. Heat shock assays were performed by diluting stationary cultures (4–5 days in YPD at 30°C) to  $1 \times 10^8$  cells/ml in water, subjecting them to 54°C for 5 min, cooling them quickly on ice and plating serial dilutions on YPD plates to determine viability.

### **CYC1 protein isolation and sequencing**

Iso-1-cytochrome *c* was isolated and sequenced as previously described (Moerschell *et al.*, 1988).

### **Northern blots and RNA probes**

RNA was isolated according to standard procedures (Nasmyth, 1983) and Northern blots were performed as previously described (Brill and Sternglanz, 1988). The  $\alpha 2/a 2$  probe, pGEM3B- $\alpha 2$ , was constructed by inserting a 2.3 kb *Xba*I–*Hind*III fragment from *MAT $\alpha$*  into the *Xba*I–*Hind*III cut pGEM3Blue riboprobe vector (Promega, Madison, WI). This plasmid was linearized with *Hind*III and anti-sense RNA was generated using T7 RNA polymerase. This riboprobe also anneals to *a 2* transcripts. The *SIR2* probe, pGEM3B-SIR2, was constructed by inserting a 2.0 kb *Bgl*III–*Hind*III fragment from the *SIR2* gene into *Bam*HI–*Hind*III cut pGEM3Blue. This plasmid was linearized with *Hind*III and anti-sense RNA was generated with T7 RNA polymerase. The *SIR2* fragment used also contains a small part of the *NAT1* gene, so riboprobes generated by T7 RNA polymerase are able to anneal weakly to *NAT1* transcripts as well as to *SIR2* transcripts.



### ***<sup>3</sup>H-Labeling of yeast histones***

Cells were grown in 100 ml YPD to  $2 \times 10^7$  cells/ml and made into spheroplasts by standard techniques (Sherman *et al.*, 1986). Spheroplasts were resuspended in 2 ml labeling buffer, incubated with 2 mCi [<sup>3</sup>H]acetate (Amersham, 2–5 Ci/mmol) according to Nelson (1982), in the presence of 100 µg/ml cycloheximide (Sigma). Pelleted spheroplasts were resuspended in ice-cold NIB (Alonso and Nelson, 1986) with a Triton X-100 concentration of 0.5%. Spheroplasts were incubated for 15–30 min on ice and pelleted (5500 g) for 8 min at 4°C. NIB incubation followed by pelleting was repeated twice. The crude nuclear pellet was resuspended in cold 100 mM Tris pH 6.8, 0.4 M NaCl, 1 mM PMSF and incubated for 10 min on ice. Nuclei were pelleted (5500 g, 8 min, 4°C) and the salt wash was repeated with a 5 min incubation on ice. Pelleted nuclei were then resuspended in 1.35 ml cold water containing 1 mM PMSF. One tenth volume (0.15 ml) of 4 N H<sub>2</sub>SO<sub>4</sub> was added while swirling and the histones were extracted on ice overnight. Slurries were centrifuged 10 min at 12 000 g at 4°C. Supernatants were transferred to 15 ml Falcon tubes, filled with cold acetone/HCl (acetone:5 M HCl, 99:1), mixed, and put at –20°C overnight. Histones were pelleted at 10 000 g for 10 min. Pellets were lyophilized and resuspended in loading buffer for Triton–acid–urea gels.

### ***Triton – acid – urea gels***

12% Acrylamide (30:0.2) gels (13 cm long, 14 cm wide, 1.5 mm thick) were made containing 8 M urea (IBI), 0.37% Triton X-100, 0.9 N acetic acid, 0.125% ammonium persulfate, and 0.125% TEMED (Alfageme *et al.*, 1974; Zweidler, 1978). After polymerization, gels were pre-electrophoresed for at least 6 h at 150 V, until amperage was constant. Wells were loaded with 1 M cysteamine (Sigma), 8 M urea, 0.9 N acetic acid and the gels were electrophoresed for another hour at 150 V. Wells were cleaned and fresh buffer was placed in both upper and lower chambers. Histones in loading buffer (8 M urea, 10% glycerol, 0.9 N acetic acid, 5% BME, 0.25% methyl green) were boiled for 5 min, cooled and loaded on gels. Histones were electrophoresed at 115–125 V for 19–21 h, until the blue component of the dye was at the bottom of the gel. Gels were stained with Coomassie Blue R (Sigma) and prepared for fluorography (Bonner and Laskey, 1974; Laskey and Mills, 1975).

### ***[<sup>35</sup>S]Methionine labeling of proteins***

Strains were grown overnight in supplemented SD medium lacking methionine and then diluted to a density of  $\sim 5 \times 10^6$  cells/ml. When the culture reached  $\sim 2 \times 10^7$  cells/ml, 1 ml of cells was labeled with 0.1 mCi of [<sup>35</sup>S]methionine (Dupont) for 15 min at 30°C. Labeled cells were transferred to an Eppendorf tube and washed twice with ice-cold water. The cells were resuspended in 0.1 ml water and an equal volume of 0.45  $\mu$ m glass beads was added. Cells were vortexed for 10 s and 0.1 ml of 0.6% SDS, 2% BME, 100 mM Tris pH 8.0 (maintained at 100°C) was added. The mixture was vortexed for 1 s and 0.1 ml was removed (avoiding glass beads) and added to an Eppendorf tube on ice containing 10  $\mu$ l RNase/DNase solution (1 mg/ml DNase, 0.5 mg/ml RNase, 500 mM Tris pH 7.0, 50 mM MgCl<sub>2</sub>). Protein samples were incubated for 1 min on ice and then frozen at  $-70^\circ\text{C}$ .