Physiological Importance and Identification of Novel Targets for the N-Terminal Acetyltransferase NatB†

Robert Caesar,* Jonas Warringer, and Anders Blomberg*

Received 15 September 2005/Accepted 16 December 2005

The N-terminal acetyltransferase NatB in Saccharomyces cerevisiae consists of the catalytic subunit Nat3p and the associated subunit Mdm20p. We here extend our present knowledge about the physiological role of NatB by a combined proteomics and phenomenics approach. We found that strains deleted for either NAT3 or MDM20 displayed different growth rates and morphologies in specific stress conditions, demonstrating that the two NatB subunits have partly individual functions. Earlier reported phenotypes of the nat3Δ strain have been associated with altered functionality of actin cables. However, we found that point mutants of tropomyosin that suppress the actin cable defect observed in nat3Δ only partially restore wild-type growth and morphology, indicating the existence of functionally important acetylations unrelated to actin cable function. Predicted NatB substrates were dramatically overrepresented in a distinct set of biological processes, mainly related to DNA processing and cell cycle progression. Three of these proteins, Cac2p, Pac10p, and Swc7p, were identified as true NatB substrates. To identify N-terminal acetylations potentially important for protein function, we performed a large-scale comparative phenotypic analysis including nat3Δ and strains deleted for the putative NatB substrates involved in cell cycle regulation and DNA processing. By this procedure we predicted functional importance of the N-terminal acetylation for 31 proteins.

A majority of all experimentally characterized eukaryotic proteins are N-terminally acetylated. In yeast, approximately 40% of the proteins are predicted to have an N-terminal acetyl group, while the corresponding figure for mammalian proteins is almost 90% (16, 19, 23). Prokaryotic proteins are rarely acetylated (4, 32). In vitro studies indicate that addition of the acetyl group occurs cotranslationally when the peptides are 30 to 50 amino acids long (4). N-terminal acetylation is catalyzed by an evolutionarily conserved group of enzymes termed N-terminal acetyltransferases (NATs). The catalytic subunits of the NATs are conserved and belong to GNAT (for “GCN5-related N-acetyltransferases”), a larger acetyltransferase family also including histone acetyltransferases (18).

In Saccharomyces cerevisiae, the model organism in which N-terminal acetyltransferases have been most extensively studied, four NATs have been identified: NatA, NatB, NatC, and Nat4p. NatA is the NAT for which most substrates have been identified (23). NatA consists of the catalytic subunit Ard1p and the associated subunits Nat1p and Nat5p. The activity of NatA is fully dependent on both Ard1p and Nat1p while the function of Nat5p appears redundant and is not fully understood (6, 17). NatC consists of the catalytic subunit Mak3p and the associated proteins Mak10p and Mak31p. All three subunits have been shown to be essential for NatC activity (22, 24).

Nat4p was recently shown to acetylate the N terminus of the histones H4 and H2A (30). No further substrates have been identified, and no associated subunits have been shown to form complex with Nat4p. NatB consists of the catalytic subunit Nat3p and the associated subunit Mdm20p (20). In previous reports, the importance of Mdm20p for NatB activity has been tested for the two NatB substrates, tropomyosin 1 (Tpm1p) and Cyc1-853, a derivative of cytochrome c. Acetylations of both proteins were shown to be Mdm20p dependent (20, 29).

With respect to potential substrates, the NAT enzymes appear nonoverlapping; i.e., they target distinct although slightly degenerate target motifs. Proteins with S, A, G, and T termini constitute potential NatA substrates, whereas proteins with M, I, L, and MF amino acid termini are potential NatC substrates. With regard to NatB, proteins with MD, ME, and MN constitute potential substrates. However, whereas MD and ME termini appear to be acetylated in 100% of the cases, only half of the experimentally investigated MN termini have been found to constitute true NatB target motifs (23).

Despite the widespread occurrence of N-terminal acetylations in eukaryotes, the biological relevance of this protein modification has only been deduced for a few substrates. In yeast, NatA-mediated N-terminal acetylations of Orc1p and of Sir3p have been shown to be necessary for transcriptional silencing (7, 33). Similarly, the loss of the NatC-mediated acetylation of the Arl-like GTPase Arl3p has been shown to abolish the targeting of Arl3p to the Golgi (1, 25). NatB-mediated acetylation of tropomyosin 1 and actin has been shown to be important for the formation of stable actin cables (20, 29). In addition, we have recently established that the cytosolic carbamoylase Y (CPY) inhibitor Tis1p is acetylated by NatB and that loss of the acetyl group results in a 100-fold decrease in CPY inhibition (3).

In this work, we investigated the overall functional importance of NatB-mediated acetylations. Using high-resolution phenotypic profiling, we report that strains deleted for either NAT3 or MDM20, the NatB subunits, display different growth
rates and morphologies in specific conditions, demonstrating that they also have individual functions. As both Mdm20p and Nat3p are required for acetylation of the previously known NatB targets Act1p, Rnr4p, and Tis1p, we conclude that the individual functions are unrelated to these modifications. We also performed a bioinformatics analysis of putative NatB substrates and found a remarkable and extreme statistical overrepresentation of proteins involved in cell cycle regulation and DNA processing, indicating that regulation of cell cycle-related processes may constitute the main functional role of NatB. These indications are supported by experimental evidence identifying three novel NatB targets, Swc7p, Pac10p, and Cac2p, all involved in cell cycle-related processes. Furthermore, we performed a large-scale comparative phenotypic analysis, including nat3Δ and strains deleted in genes coding for proteins involved in cell cycle regulation and DNA processing, and predict functional importance of the N-terminal acetyl group for 31 proteins.

**MATERIALS AND METHODS**

**Strains, media, and growth conditions.** *S. cerevisiae* strains used in this study are listed in Table 1. pRS316 carrying *TPM1* mutations was kindly provided by Bogdan Polevoda. All strains were cultured at 30°C in succinate-buffered synthetic medium (optical density at 610 nm [OD610] of approximately 4.0) by centrifugation at 4,000 rpm at 4°C. Protein extracts were prepared by glass bead protein extraction by sonication in urea-thiourea buffer and isoelectric focusing.

**TABLE 1. S. cerevisiae strains**

| Description | Strain background | Genotype | Reference or source |
|-------------|------------------|----------|---------------------|
| Wild type   | BY4742           | MATa his3Δ1 leu2Δ200 ura3Δ0 lys2Δ0 | EUROSCARF |
| Deletion strain* | BY4742 | MATa his3Δ1 leu2Δ200 ura3Δ0 lys2Δ0 nat3Δ3; KanMX4 p[CEN URA3] | This work |
| nat3Δ control plasmid | BY4742 | MATa his3Δ1 leu2Δ200 ura3Δ0 lys2Δ0 nat3::CEN-MX4 p[CEN URA3] | This work |
| nat3Δ TPM1-5 | BY4742 | MATa his3Δ1 leu2Δ200 ura3Δ0 lys2Δ0 nat3::CEN URA3 p[CEN URA3-TPM1-5] | This work |
| nat3Δ TPM1-4 | BY4742 | MATa his3Δ1 leu2Δ200 ura3Δ0 lys2Δ0 nat3::CEN URA3 p[CEN URA3-TPM1-4] | This work |
| Wild-type control plasmid | BY4742 | MATa his3Δ1 leu2Δ200 ura3Δ0 lys2Δ0 nat3::CEN URA3 | This work |
| Wild-type TPM1-5 | BY4742 | MATa his3Δ1 leu2Δ200 ura3Δ0 lys2Δ0 nat3::CEN URA3 p[CEN URA3-TPM1-5] | This work |
| Wild-type TPM1-4 | BY4742 | MATa his3Δ1 leu2Δ200 ura3Δ0 lys2Δ0 nat3::CEN URA3 p[CEN URA3-TPM1-4] | This work |
| Wild-type GFP fusionsb | BY4741 | MATa his3Δ1 leu2Δ200 met15Δ0 ura3Δ0 X-GFP-His3MX; X = ioc3 swi3 sp8 gsi1 yph1 dst1 syf2 kar9 kar3 thp1 mle2 bud3 | 11 |
| Wild-type TAP fusionsb | BY4741 | MATa his3Δ1 leu2Δ200 met15Δ0 ura3Δ0 nat3::CEN-MX4; X-GFP-His3MX; X = ioc3 swi3 sp8 gsi1 yph1 dst1 syf2 kar9 kar3 thp1 mle2 bud3 | 9 |
| nat3Δ GFP fusionsb | BY4741 | MATa his3Δ1 leu2Δ200 met15Δ0 ura3Δ0 nat3::CEN-MX4; X-GFP-His3MX; X = ioc3 swi3 sp8 gsi1 yph1 dst1 syf2 kar9 kar3 thp1 mle2 bud3 | X-GFP-His3MX; X = pac10 pin4 swc7 cac2 cass5 cin2 mle2 hda3 pph21 |
| nat3Δ TAP fusionsb | BY4741 | MATa his3Δ1 leu2Δ200 met15Δ0 ura3Δ0 nat3::CEN-MX4; X-TAP-His3MX; X = pac10 pin4 swc7 cac2 cass5 cin2 mle2 hda3 pph21 | X-GFP-His3MX; X = pac10 pin4 swc7 cac2 cass5 cin2 mle2 hda3 pph21 |

* X denotes deleted genes.

b X denotes fused genes.

by PCR using primers with the 5′ to 3′ sequences TCCCATCCTTTTCGTA GTTGGTTG and ACCGACCTTTAGTGCAGCATCAT (internal KanMX4 marker sequence). Linear transformation was performed as described elsewhere (31). Plasmid transformation was performed using the lithium acetate/polycytethylene glycol method.

**Inhibitors.** All inhibitors used were of the highest available grade (Sigma-Aldrich). Concentrations were as follows: NaCl, 0.85 M; methyl methanesulfonate, 0.0015%; clotrimazole, 1.5 mM; diamide, 1.4 mM; dithiothreitol (DTT), 1.6 mM; tunicamycin, 1 µg/ml; hydroxyurea, 8 mg/ml; 6-azauracil, 200 µg/ml; ketoconazole, 20 µM; KCl, 1.45 M; CdCl, 247.5 µM; MnCl, 210 µM; LiCl, 100 mM; ethidium bromide, 45 µg/ml; 2,4-dinitrophenol (DNP), 0.2 mg/ml; cycloheximide, 0.05 µg/ml; 1,10-phenanthroline, 2 µM; tert-butyl hydrogenperoxide (t-butylHOOH), 0.35 mM; cerulenin, 0.22 µg/ml; cephalomon, 0.05 µg/ml; myriocin, 2 µg/ml; 2,3 galactose, hygromycin B, 1.25 mg/ml; vanadat, 0.47 mM; caffeine, 1 mg/ml; parguatoq, 300 mg/ml; paramomycin sulfate, 12 mg/ml; triphenethiazine, 50 µM; thiabendazole, 0.05 µg/ml; doxorubicin, 10 µg/ml; nalidixic acid, 0.05 mg/ml; etoposide, 100 µg/ml; 2,3 butandione monoxime, 25 µM; cispitant, 100 µg/ml; fenpropimorph, 0.05 mg/ml; 4-nitroquinolone (4NQO), 0.24 µg/ml; dimethyl sulfoxide (DMSO), 3%; anisomycin, 0.5 µg/ml; rapamycin, 0.6 µg/ml.

**Two-dimensional electrophoresis (2D-PAGE).** 2D-PAGE was performed as previously described (2). Briefly, 5–ml cell cultures were harvested in early stationary phase (optical density at 600 nm [OD610] of approximately 4.0) by centrifugation at 4,000 rpm at 4°C. Protein extracts were prepared by glass bead disruption, and 150 µg protein was loaded on each gel. Proteins were visualized by silver staining.

**Isoelectric focusing and Western blot analysis.** Ten milliliters of cell culture was harvested in mid-maxponential phase (OD610, 0.5 to 1.0). Procedures for protein extraction by sonication in urea-thiourea buffer and isoelectric focusing were as previously described (2), using nonlinear 3 to 10 or linear 4 to 7–immobilized pH gradient strips (Amersham Bioscience).

After isoelectric focusing, strips were equilibrated in a buffer containing 0.1 M sodium dodecyl sulfate, 0.05 M DTT, and 0.35 M Tris for 30 min and subsequently in transfer buffer (0.1 M glycine, 10 mM Tris base, and 20% methanol) for 10 min. The protein backing was removed from the strips, and the proteins were transferred to polyvinylidene difluoride membranes on Multisorb II semi-dry blotting equipment (Amersham Bioscience) according to manufacturer protocols. Membranes were blocked in TBST (10 mM Tris, 0.15 M NaCl, 0.05 M Tween 20, pH 8) supplemented with 5% skim milk, washed twice for 15 min in TBST, incubated overnight in anti-calmodulin antibodies (0.07–0.42; Upstate Biotechnologies) diluted 1:500 in TBST–5% skim milk, washed twice for 15 min in TBST, incubated for 1 h in anti-rabbit secondary antibodies (NHB934V; Amersham Bioscience) diluted 1:4,000, and finally washed three times for 15 min in TBST.
Membranes were incubated for 5 min in ECL Plus Western blotting detection solution (RPN2132; Amersham Bioscience), and the signal was recorded using a Fujifilm Dark Box II.

**Statistical analysis of putative NatB targets.** The distribution of functions among putative NatB targets was compared to the distribution of functions among all *S. cerevisiae* proteins using functional annotations taken from MIPS (http://mips.gsf.de/genre/proj/yeast). Functional annotations were investigated at the highest possible level of resolution. To maintain statistical validity, the highest level of resolution was limited to classes containing at least 15 members. The statistical significance of functional overrepresentations among NatB targets was determined for each functional class separately, assuming Poisson distribution of the data, i.e., the standard deviation of each functional class was approximated as the square root of the expected mean. The probability of overrepresentation was determined for each functional class separately, assuming Poisson distribution of the data, i.e., the standard deviation of each functional class was approximated as the square root of the expected mean. The probability of overrepresentation was calculated assuming a normal distribution. Proteins with leader peptide were not excluded from the analysis, since leader peptides may be of importance for, e.g., localization.

The distribution of localization was similarly investigated.

**Bright-field and fluorescence microscopy.** Cells were studied during exponential phase growth (OD_{610}, 0.5 to 1.0). Microscopy was performed on a Leica DM R microscope.

**Calculation of growth variables and growth ratios.** Growth rate was calculated as described elsewhere (34). To quantify the growth rate of each strain compared to a reference strain, a logarithmic coefficient, LSC, was calculated. The LSC value describes the growth rate of the mutant in relation to the growth rate of the wild type, with negative values indicating reduced growth. LSC is calculated by subtracting the value of the logarithmized growth rate of the deletion strain from the value of the logarithmized growth rate of the wild type (35).

Logarithmic phenotypic indexes, LPI, which describe the sensitivity of the strain to a specific inhibitor, were calculated according to the following equation: LPI = LSC_{mutant} − LSC_{basal}.

Statistical significance was calculated using a Student’s *t* test as previously described (35). In order not to reject the null hypothesis only because of low variance by chance, a threshold value of 0.1 was applied. In all cases this gave a combined significance of *P* < 0.001.

**RESULTS**

The NatB subunits exhibit partly divergent phenotypes. In a previous report we provided evidence that a strain lacking Nat3p, the catalytic subunit of NatB, has reduced growth rate, reaches lower population densities, and exhibits abnormal morphology, including multiple buds and increased cell size, in minimal medium (3). Recently, Nat3p has been shown to bind to Mdm20p, forming a functional complex (20). To investigate the dependence of NatB on Mdm20p, we performed a quantitative phenotypic comparison between *mdm20A* and *nat3Δ*. With this high-resolution phenotyping methodology (35), we analyzed growth defects under close to optimal physiological conditions (roughly 50% growth inhibition). First, strains lacking Nat3p or Mdm20p were cultivated in minimal medium with no external stress (Fig. 1A). Surprisingly, strains lacking Mdm20p did not resemble strains lacking Nat3p in control medium, neither in terms of the growth rate and/or efficiency nor in cell morphology. Rather, *mdm20A* exhibited unperturbed growth and was indistinguishable from the reference strain. Second, cells were cultivated in the presence of 19 inhibitors of different distinct biological processes. To provide a measure of the specific gene-by-environment interaction, logarithmic phenotypic indexes (LPI) (35) that normalize for growth defects under normal conditions were calculated; this is highly relevant, since the *nat3Δ* strain grows much more slowly in control conditions. Strains lacking *mdm20A* or *nat3Δ* displayed similar gene-by-environment interactions in the presence of most inhibitors (Fig. 1B). Both strains were significantly sensitive to, e.g., the DNA-damaging agents hydroxyurea, ethidium bromide, and 4-nitroquinoline (4-NQO), ion stress exerted by KCl, and myriocin-mediated perturbations of the sphingolipid biosynthesis. However, they displayed remarkable differences in growth during treatment with a few specific compounds; *nat3Δ*, in contrast to *mdm20A*, was found to be sensitive to the cyclic AMP phosphodiesterase inhibitor caffeine, whereas *mdm20A*, in contrast to *nat3Δ*, was sensitive to LiCl and DMSO. The differences observed between strains lacking Nat3p and Mdm20p, both in terms of morphology and growth, provide strong evidence that the subunits possess some function(s) distinct from each other.

**Mdm20p is essential for the acetylation of Act1p, Rnr4p, and Tfs1p.** To determine whether the observed differences in morphology and growth between strains lacking Nat3p and Mdm20p are associated with the N-terminal acetyltransferase activity of the NatB complex, we compared acetylation patterns in *mdm20A*, *nat3Δ*, and wild-type cells. The loss of acetylation on substrate proteins in NAT deletion strains is typically characterized by a shift to more alkaline values in the isoelectric point. Comparing 2D-PAGE gels with whole-cell protein extracts from the wild type and *nat3Δ* (3, 21), we have previously identified three NatB substrates, Act1p, Rnr4p, and Tfs1p. Using the same approach, we here report that *mdm20A* and *nat3Δ* display identical acetylation patterns; the three known NatB targets were completely dependent on Mdm20p for acetylation (Fig. 2). In addition, no other proteins with altered isoelectric points could be detected. We conclude that the observed differences in morphology, growth, and stress tolerance between *mdm20A* and *nat3Δ* strains are not due to the loss of the functionally important acetyl groups on Act1p or Tfs1p or to the loss of acetylation on Rnr4p.

**Restoring actin filaments does not fully suppress the NatB phenotype.** Both *nat3Δ* and *mdm20A* cells have been shown to lack stable actin filaments, a feature caused by the loss of acetylation of tropomyosin 1 and/or actin (10, 29) and believed to be highly important in setting the phenotypes of these strains. However, the here-reported similar defects on actin acetylation from deleting either of the two NatB subunits indicates that other proteins, unrelated to actin filament formation, strongly contribute to the phenotype of the *NAT3* and the *MSM20* gene deletions. To further investigate the contribution of the actin filament defect to the overall *nat3Δ* phenotype, *nat3Δ* cells and wild-type cells were transformed with plasmids carrying the tropomyosin 1 mutant *TPM1*+5. This mutation has previously been shown to restore actin filaments in the *nat3Δ* mutant (28, 29). The *TPM1*+5 mutant exhibited a small but consistently positive effect on the *nat3Δ* phenotype in the presence of a wide range of growth inhibitors (Fig. 3B). The *TPM1*+5 mutant exhibited a significant (*P < 0.001*) positive effect on the stress sensitivity of *nat3Δ* in 31 of 33 tested environments (Fig. 3C), with the average increase in stress tolerance in terms of rate of growth being 18%. However, in no single environment did the *TPM1*+5 mutation completely restore wild-type growth to the *nat3Δ* strain. Furthermore, the *TPM1*+5 mutant did not suppress the morphology defect of *nat3Δ* (Fig. 3B).

To confirm these observations, the experiment was repeated with a second tropomyosin 1 mutant, *TPM1*−4, earlier reported to restore actin filaments in *mdm20Δ* (28). The results thus derived closely resembled the results from the *TPM1*+5 experiment (data not shown). Hence, we conclude that even though
the actin cytoskeleton defects of strains lacking Nat3p are negated by insertion of mutant tropomyosin, at large the observed growth defects of nat3Δ remains. This indicates that contrary to what has earlier been proposed (20), the growth defect of nat3Δ is not predominantly caused by defects in the actin cytoskeleton.

Putative NatB substrates are strongly overrepresented in functional groups involved in cell cycle progression and DNA maintenance. What then could be the cause of the main phenotypes of NatB mutants? NatB has been found to acetylate substrates with the N-terminal amino acid sequences ME, MD, and MN. In the case of proteins with N-terminal ME and MD, all 15 experimentally investigated proteins have been found to be acetylated (14, 23). Hence, it is not unreasonable to assume that many, if not most, of the 589 yeast proteins, i.e., 10% of the yeast proteome, with an N-terminal methionine followed by an acidic penultimate amino acid residue are indeed acetylated by NatB. In light of this observation, it is surprising that only three of over 600 proteins detected on 2D-PAGE gels, i.e., less than 0.5%, exhibit a shift in pI on nat3Δ gels. As only

![Graph A](image1.png)

**FIG. 1.** Phenotypes for nat3Δ and mdm20Δ. (A) Growth in basal medium. Filled squares represent wild-type culture, open circles represent mdm20Δ culture, and open squares represent nat3Δ culture. Microscopy pictures are not in the same scale. (B) Logarithmic phenotypic index (LPI) values for growth rate. White bars represent nat3Δ, and black bars represent mdm20Δ. Two stars indicate significance ($P < 0.001$) as defined in Materials and Methods.

![Graph B](image2.png)

**FIG. 2.** Portions of 2D-PAGE gels of protein extracts prepared from wild-type, nat3Δ, and mdm20Δ cells. The positions of the acetylated and the unacetylated proteins are indicated by circles in each picture. Due to the loss of the N terminus the acetyl group proteins are moved toward the basic end of the gel in the deletion mutant strains.
the most highly expressed proteins are detectable on 2D gels, this raises the concern that NatB targets may be underrepresented among highly expressed proteins. To investigate this hypothesis, we compared the codon adaptation index (CAI) of proteins with ME and MD termini to the CAI of all proteins. We found ME and MD terminus proteins to be strongly underrepresented among proteins with a high CAI (Fig. 4A; most clearly seen for CAI > 0.6), thus confirming the hypothesis that NatB targets are less expressed than proteins in general. Highly expressed proteins tend to belong to a very distinct set of functional categories, notably energy production and protein synthesis (12). Hence, it may be hypothesized that the underrepresentation of NatB targets among highly expressed proteins reflects an underlying preference of NatB to acetylate...
certain functional classes of proteins. To reject or confirm this hypothesis, we compared the functional annotation of the putative NatB targets with ME and MD termini to that of yeast proteins in general. The presumed NatB substrates in the ME- and MD-terminus class displayed a remarkably uneven distribution with regard to biological process annotation. Predicted NatB substrates tended to be overrepresented in a very distinct set of biological processes, mostly encompassing functions related to DNA processes as well as cell cycle progression. Most significant (P < 0.001) were the overrepresentations in subprocesses such as nuclear and chromosomal cycle, chromosomal segregation, and division and biogenesis of the nucleus (Fig. 4B, Table S2 in the supplemental material). The indicated enrichments correspond to 1.6- to 2.9-fold overrepresentations, with the highest overrepresentations observed for chromosomal segregation and division. The preference of NatB substrates to participate in DNA processing and cell cycle progression functions were also reflected in a high tendency of NatB substrates to be localized to the tubulin cytoskeleton, the spindle pool body, and the chromosomal structure. We also noted a high overrepresentation of NatB targets in intracellular transport vesicles (Fig. 4C). To ensure that the observed enrichments were not an indirect consequence of the NatB preference for low-abundance proteins, functional overrepresentations among low-abundance proteins (CAI < 0.2) were also investigated separately. None of the functional categories overrepresented among NatB targets were overrepresented among low-abundance proteins (1.15-fold enrichments or lower). These findings strongly indicate that NatB preferentially acetylates proteins involved in certain cellular processes, notably cell cycle progression and DNA processing. An attractive hypothesis would be that some of these NatB substrates have coevolved with NatB.

Swc7p, Cac2p, and Pac10p are novel NatB substrates. To investigate whether the putative NatB substrates in the cell cycle control and DNA processing functional categories constitute true NatB substrates, a subset of the NatB substrates was investigated experimentally. Ten highly expressed tandem affinity purification (TAP)-tagged proteins (9) with predicted near-neutral pI, Pac10p, Pin4p, Swc7p, Cac2p, Csm1p, Sas5p, Cin2p, Mlc2p, Hda3p, and Pph21p, were introduced into nat3 and wild-type cells, and protein extracts were subjected to isoelectric focusing followed by Western blot using anti-calmodulin antibodies detecting TAP tags. Seven of the 10 TAP-tagged proteins did not give a clear and reliable signal; however, the remaining three, Swc7p, Cac2p, and Pac10p, yielded a good signal, and all exhibited a distinct shift in focusing position towards the basic side in the nat3 strain, indicating loss of an N-terminal acetyl group (Fig. 5). These three proteins were found at pIs corresponding to their theoretical pIs. We conclude that Swc7p (N-terminal MD), Cac2p (N-terminal ME), and Pac10p (N-terminal MD) constitute novel NatB substrates. Even if we, by this procedure, increased the number of confirmed NatB substrates by only three, we see this as further evidence that the NatB sequence requirements are conserved, and we believe most of the remaining putative targets in the cell cycle and DNA processing class to be true NatB substrates.

NatB-mediated acetylation is not a general signal for protein localization. The high overrepresentation of putative proteins with MD or ME as N-terminal residues within categories of (B) cellular functions and (C) localization. Numbers given after category names represent fold enrichments. Overrepresentation corresponds to a P value of 0.001.

FIG. 4. Codon adaptation index (CAI) and overrepresentation in functional and localization categories of proteins with MD and ME as N-terminal residues. (A) Representation of all S. cerevisiae proteins (black bars) and protein with MD and ME as terminal residues (white bars) within given CAI intervals. Significance of enrichment of proteins with MD or ME as terminal residues within categories of (B) cellular functions and (C) localization. Numbers given after category names represent fold enrichments. Overrepresentation corresponds to a P value of 0.001.
NatB substrates localized in, e.g., chromosome structure and spindle pool bodies (Fig. 4C) suggests that acetylation may be of importance for protein localization. Thirteen proteins in the cell cycle control and DNA processing functional category were selected, and proteins fused with C-terminal green fluorescent protein tags were expressed in nat3Δ and wild-type cells. Protein localization was determined using fluorescence microscopy. Eight proteins, Ioc3p, Swi3p, Spt8p, Gis1p, Yhp1p, Pgd1p, Dst1p, and Syf2p, localized in the nucleus; two proteins, Kar9p and Kar3p, localized in the spindle pool bodies; one protein, Thp1p, localized in the nucleus periphery; and two proteins, Mic2p and Bud3p, localized at the bud neck, were included in the study. None of the 13 proteins showed any difference in localization between wild-type and nat3Δ cells. All proteins exhibited easily determined localization in both strains (data not shown), thus most likely excluding a mislocalization of nonacetylated proteins as an explanation for the observed phenotypes. Protein localization was in accordance with previous reports on wild-type protein localization (11).

Phenotypic analysis of gene deletions of proposed NatB substrates revealed proteins with potentially functionally important acetylations. Strains lacking Nat3p displayed defects in tolerance to a range of environmental stresses (Fig. 1). To deduce whether these nat3Δ defects could be due to the lack of acetylation leading to loss of function of substrates involved in cell cycle progression and DNA processing events, we performed a comparative phenotypic screen of nat3Δ and all 84 viable disruption mutants that correspond to predicted NatB substrates involved in these processes. Provided that defects in the NatB-derived acetylation render a substrate nonfunctional (or strongly hamper functionality), we expected to see similarities in phenotypic behavior between nat3Δ and strains deleted for the specific substrate protein.

An extreme possibility may be that NatB has evolved mainly to acetylate one prime target involved in cell cycle progression and DNA processing. If this is the case, we expect nat3Δ to display phenotypes very similar to those of a strain deleted for this single substrate. Hence, we determined the overall phenotypic similarity between nat3Δ and the strains deleted in genes coding for predicted NatB substrates by hierarchical clustering of all derived quantitative phenotypes in the 30 different environments. However, no intimate clustering could be found between nat3Δ and any of the other strains included in the screen when considering all conditions (data not shown). We conclude that the stress defects of the nat3Δ strain are caused by defects in the acetylation patterns of many substrates and are not due to the lack of acetylation of a single substrate that is fully dependent on acetylation.

However, only including the nine conditions where nat3Δ displayed a significant phenotype, a number of deletions strains clustered close to nat3Δ (Fig. 6A). The strain most similar to nat3Δ is deleted for the gene THPI, encoding a protein involved in transcription elongation and mitotic recombination. Similar observations could be made for pac11Δ, arp1Δ, and pan3Δ, which also group together with nat3Δ; however, the magnitude of phenotypic change for these deletion strains was mostly much smaller than for nat3Δ.

When gene redundancy and compensatory pathway mechanisms are in operation, we would expect functionally important NatB substrates to display similar phenotypes to nat3Δ only under a limited number of conditions, maybe only one. We thus identified potentially acetylation-dependent proteins by scoring the most pronounced phenotypes in all single conditions where nat3Δ displayed a phenotype. Applying this logic, we extended the list of NatB substrates that are potentially acetylation dependent for functionality to include 31 proteins (see Table S1 in the supplemental material). The stress defects most easily linked to cell cycle progression and DNA processing are defects in the tolerance to hydroxyurea and ethidium bromide. We found strains deleted for three predicted NatB targets, red1Δ, mnt2Δ, and sfp1Δ, to exhibit significant defects in the tolerance to both these environmental stresses in a manner closely resembling that of nat3Δ (Fig. 6B). The most extreme nat3Δ sensitivity was recorded in the presence of 2,3-butanedione 2-monoxime (BDM) (a more than twofold prolonged generation time compared to the defect in the wild type). Interestingly, Pac10p, identified as a NatB substrate in this study (Fig. 5), also exhibited a significant phenotype with this compound. The BDM phenotype may partially be linked

---

**FIG. 5.** Western blot of isoelectrically focused proteins. Proteins fused with C-terminal TAP tags extracted from wild-type and from nat3Δ cells. Due to the loss of the N-terminal acetyl group, proteins in nat3Δ/H9004 and wild-type/H9004 exhibit similar isoelectric points. The extreme NatB substrates localized in, e.g., chromosome structure and spindle pool bodies (Fig. 4C) suggests that acetylation may be of importance for protein localization. Thirteen proteins in the cell cycle control and DNA processing functional category were selected, and proteins fused with C-terminal green fluorescent protein tags were expressed in nat3Δ and wild-type cells. Protein localization was determined using fluorescence microscopy. Eight proteins, Ioc3p, Swi3p, Spt8p, Gis1p, Yhp1p, Pgd1p, Dst1p, and Syf2p, localized in the nucleus; two proteins, Kar9p and Kar3p, localized in the spindle pool bodies; one protein, Thp1p, localized in the nucleus periphery; and two proteins, Mic2p and Bud3p, localized at the bud neck, were included in the study. None of the 13 proteins showed any difference in localization between wild-type and nat3Δ cells. All proteins exhibited easily determined localization in both strains (data not shown), thus most likely excluding a mislocalization of nonacetylated proteins as an explanation for the observed phenotypes. Protein localization was in accordance with previous reports on wild-type protein localization (11).
to the reported actin defect of nat3Δ (we note, for example, that the TPM1-5 mutant decreases the nat3Δ sensitivity to BDM by approximately 30% [Fig. 3B]) and may constitute a compound phenotype resulting from defects in actin functionality and the acetylation defect of other substrates. The BDM phenotype was most pronounced in the strains csm1Δ, red1Δ, nnf2Δ, and thp1Δ.

We conclude that the overall phenotype of nat3Δ could include several of the here-proposed substrates in the cell cycle or DNA processing categories as well as the earlier proposed

FIG. 6. Phenotypic similarity between nat3Δ and strains deleted for putative NatB targets. (A) Hierarchical clustering of nat3Δ and strains deleted for putative NatB targets. The strains were subjected to average linkage hierarchical clustering using an uncentered Pearson correlation coefficient as a similarity measurement. (B) LPI values for growth rate for nat3Δ (black bars) and for strains deleted in genes coding for putative NatB substrates (white bars) exhibiting significant sensitivity to hydroxyurea and ethidium bromide.
defect in actin functionality. Our phenotypic screen presents potential candidates for functional NatB-mediated acetylations. Further biochemical characterization focused on each of the candidates will be needed to firmly associate these acetylations with protein function.

**DISCUSSION**

**NatB acetylations in a broad physiological perspective.** Although nat3Δ and mdm20Δ phenotypes likely constitute a composite of effects from faulty acetylation of a multitude of substrates, these targets may very well be tightly linked on a functional level. Such a link would indicate that NatB has coevolved with a specific target process rather than with single substrates. Here we found support for such an evolutionary hypothesis by the observation that predicted NatB substrates with ME and MD termini follow a highly skewed distribution with regard to both function and localization (Fig. 4). Whereas proteins located at the mitochondria or involved in, e.g., protein synthesis, ribosome biogenesis, and energy very seldom constitute NatB targets, proteins involved in cell cycle progression and DNA processing are highly overrepresented among predicted NatB targets. This enrichment suggests that NatB may have evolved to fulfill a function in DNA processing, an assumption supported by the pronounced sensitivity of strains lacking NatB components both to DNA damaging drugs and, as earlier reported, to X-ray radiation (5).

To exclude the possibility that the overrepresentation of proteins involved in cell cycle progression and DNA processing among predicted NatB targets constitutes artifacts (i.e., that the earlier determined rules for NatB specificity would be nonuniversal), we investigated the most highly expressed predicted targets in this functional category experimentally. We found that the predicted targets expressed at detectable levels in our assay indeed constituted true targets of NatB acetylation. Hence, we not only conclude that the observed preference for NatB to acetylate proteins involved in cell cycle progression and DNA processing constitute a real biological phenomena, we also extend the list of known NatB substrates with ME or MD termini from 15 to 18. We note that these 18 substrates display a bewildering variation in the third and forth amino acids, including amino acids with small, large, polar, nonpolar, acidic, basic, and neutral side chains. From this observation we predict that a vast majority of the 589 ME- and MD-terminus proteins in yeast constitute true NatB targets, although the acetylations may not in all cases be functionally important.

**The role of Mdm20p in NatB.** Mdm20p was initially identified as a protein essential for tropomyosin-F-actin interaction and thereby stabilization of actin cables (10). It was later found that Mdm20p forms a complex with Nat3p (20) and that both Mdm20p and Nat3p are required for the acetylation of Tpm1p and for the formation of stable actin cables (29). Actin itself is also a NatB substrate (21), and phenotypic features of the actin mutant ACT1-136, mimicking the properties of an unacetylated actin, suggest that the acetylation of actin is also important for actin cable formation (20). In this paper, we show that although nat3Δ and mdm20Δ share some features, including the requirement for both Nat3p and Mdm20p to acetylate actin, they also display many distinct phenotypes which are not found for strains lacking the other component of the NatB complex. Notably, mdm20Δ does not exhibit the slow growth characteristic in minimal medium of nat3Δ and also differs from nat3Δ in stress tolerance (Fig. 1B). Phenotypic differences between nat3Δ and mdm20Δ have previously been observed in both BY (35) and in FY backgrounds (29). This puts NatB in stark contrast to the other two major acetyltransferases in S. cerevisiae, NatA and NatC, where we can find no significant phenotypic differences between the functional subunits (unpublished data). The observation that both mdm20Δ and nat3Δ fail to acetylate not only Tpm1p but also Act1p suggests that these phenotypic differences must be due to other cellular defects than the reported defect in actin cable organization. It could be suggested that (i) Mdm20p is not necessary for the acetylation of all NatB substrates, (ii) Mdm20p is toxic for the cell in the absence of Nat3p, or, as previously suggested (29), (iii) Nat3p and/or Mdm20p possesses other functions than acetyltransferase activity. We here report the novel observation that Act1p, Rnr4p, and Tfs1p all are dependent on Mdm20p for acetylation. These three proteins have the N-terminal amino acid sequences MDSEV, MEAHN, and MNQAI, respectively. In addition, Tpm1 (MKDIK) and the Cyc1p derivate Cyc1-853 (MEFLA) have previously been shown to rely on Mdm20p to be acetylated (20, 29). Hence, substrates with the penultimate amino acids D, E, and N have all been shown to be dependent on Mdm20p for acetylation, which indicates that alternative (iii) above, i.e., that either Nat3p or Mdm20p has additional functions besides acetyltransferase activity, at the moment seems most likely. The individual functions of the NatB subunits certainly deserve to be further investigated.

**Candidates for functional NatB acetylations.** A fundamental question is whether NatB has evolved mainly to acetylate one or a few substrates, i.e., if only a few of the multitude of acetylations are indeed functional and account for all the defects observed in strains deleted for NatB components or if NatB has evolved to acetylate a variety of targets, each of which contributes to a subset of the phenotypes observed in nat3Δ. To resolve this question, we compared the phenotypes of nat3Δ to those of strains deleted for predicted substrates involved in cell cycle progression and DNA processing implicated by functional enrichment to constitute the most biologically relevant NatB targets. Strains carrying proteins with altered N-terminal amino acid sequences, unable to be acetylated, would possibly have been more appropriate to compare to nat3Δ. However, no collection of such strains is available. Our screen with deletion strains suffers from the limitation that only proteins that are fully, or almost fully, dependent on their N-terminal acetylation will share the phenotype of nat3Δ. We found that none of the investigated deletion strains displayed a complete or close to complete phenotypic similarity to the strain deleted for NAT3 or could account for all the observed nat3Δ phenotypes. Thus, we conclude that nat3Δ phenotypes are not the consequence of faulty acetylation of a singular target involved in cell cycle progression or DNA processing, which is unlikely given the reported important role of actin acetylation. Rather, as suggested by the small but consistent rescue of many of the observed phenotypes by the TPM1-5 mutant, the different nat3Δ defects results from the loss of acetylations in many functional substrates.
To identify which nat3Δ phenotype corresponds to which NatB substrate, we compared the nat3Δ strain to strains deleted for components in the cell cycle progression and DNA processing machinery individually. We found that the pronounced defects of nat3Δ in the tolerance to the DNA-damaging drugs hydroxyurea and ethidium bromide resembled the growth defects of three deletion strains: red1Δ, mnf2Δ, and sfp1Δ. As sfp1Δ, in contrast to red1Δ and mnf2Δ, which displayed numerous phenotypes not found for nat3Δ, only had one additional phenotype, we conclude that Sfp1p constitutes a more likely candidate for acetyl group functionality. thpΔ, the strain that clustered most closely to nat3Δ, shares all the significant phenotypes of nat3Δ except sensitivity to ethidium bromide and KCl. Even though it also exhibited a number of other phenotypes, it is indeed a highly interesting candidate for further investigation of acetyl group functionality. The protein Est1p is also interesting as a candidate for acetyl group dependency. A strain deleted for EST1 is highly sensitive to ethidium bromide and caffeine, and like nat3Δ it also exhibits sensitivity against BDM and hygromycin.

The only marginal suppression of the nat3Δ stress phenotypes by TPM1-5 and TFM1-4 mutants, which essentially completely negates the actin cable defect of strains lacking Nat3p (29), suggests that the nat3 growth defects are not, as previously proposed (20), mainly dependent on the faulty NatB acetylation of Act1p and Tpm1p. Rather, it suggests that the different nat3Δ phenotypes are caused by defects in the acetylation pattern of different substrates and that even individual phenotypes may be due to the lack of multiple acetylations. Previous growth measurements of nat3Δ using qualitative plate assays indicate a stronger correlation between, e.g., the KCl phenotype and actin defects (20). However, these earlier results do not take into account the strong growth defect of nat3Δ in basal minimal media, something which greatly influences the phenotypic results for strains like nat3Δ that grow slowly even without any stress applied. It should also be emphasized that we have cultivated cells in liquid, synthetic media, while rich, solid media has been used in previous reports. Hence, differences between our results and results presented by others could be due to differences in experimental setup.

Three novel NatB substrates. In this work three novel NatB substrates, Cac2p, Pac10p, and Swc7p, were identified. Cac2p constitutes an evolutionarily conserved component of the chromatin assembly factor CAF-1, involved in histone assembly onto recently replicated DNA (13), and is also a building block of the kinetochores (26). Swc7p is also involved in histone assembly, as it is a subunit of the Sm2 family ATPase SWT1, which is responsible for the recruitment of the histone H2AZ, a variant of H2A, into nucleosomes (15). It could be hypothesized that the acetylation for these substrates plays the role of blocking the N-terminal positively charged amino group providing less N-terminal interaction with the negatively charged phosphate backbone of DNA. The third confirmed NatB substrate, Pac10p, is a subunit of the cochaperone complex GimC, which interacts with the chaperonin TRiC to promote efficient folding of actin (27) and tubulin (8). Strains deleted for PAC10 exhibit microtubule disassembly at low temperature.

The importance of the N-terminal ends of these NatB substrates has not been investigated, and we have no firm biochemical or structural information as to whether these acetylations are functional. In the phenotype screen presented in this work, we found swc7Δ to be sensitive to diamide, pac10Δ to be sensitive to 6-azauracil, KCl, cyclohexamide, and BDM, and cac2Δ to be sensitive to DTT, hygromycin, cerulenin, and 2,4-DNP. It is noteworthy that nat3Δ also exhibited sensitivity to many of these growth inhibitors, possibly indicating the functionality of Swc7p, Pac10p, and Cac2p to be dependent on their N-terminal acetylation.

ACKNOWLEDGMENTS

This work was conducted with financial support by SSF (The Swedish Strategic Research Foundation).

The help with analysis of growth data in the PROPHECY database by Luciano Fernandez-Ricaud and the laboratory assistance by Ellinor Pettersson are highly appreciated.

REFERENCES

1. Behnia, R., B. Panic, J. R. Whyte, and S. Munro. 2004. Targeting of the Arf-like GTPase Arl3p to the Golgi requires N-terminal acetylation and the membrane protein Sys1. Nat. Cell Biol. 6:405–413.
2. Blommberg, A. 2002. Use of two-dimensional gels in yeast proteomics. Methods Enzymol. 350:559–584.
3. Caesar, R., and A. Blommberg. 2004. The stress-induced Ts1p requires NatB-mediated acetylation to inhibit carbamoylase phosphate and to regulate the purine kinase A pathway. J. Biol. Chem. 279:38532–38543.
4. Driessen, H. P., W. W. de Jong, G. I. Tesser, and H. Bloemendal. 1985. The mechanism of N-terminal acetylation of proteins. CRC Crit. Rev. Biochem. 18:281–320.
5. Game, J. C., M. S. Williamson, and C. Baccari. 2005. X-ray survival characteristics and genetic analysis for nine Saccharomyces deletion mutants that show altered radiation sensitivity. Genetics 169:51–63.
6. Gautschi, M., S. Just, A. Mun, S. Ross, P. Rucknagel, Y. Dubauique, A. Ehrenhofer-Murray, and S. Rospert. 2003. The yeast Nα-acetyltransferase Nata is quantitatively anchored to the ribosome and interacts with nascent polypeptides. Mol. Cell. Biol. 23:7403–7414.
7. Geissenhoner, A., C. Weise, and A. E. Ehrenhofer-Murray. 2004. Dependence of ORC silencing function on NatA-mediated N-acetylation inSaccharomyces cerevisiae. Mol. Cell. Biol. 4:10300–10312.
8. Geissler, S., K. Siegers, and E. Schiebel. 1998. A novel protein complex promoting formation of functional alpha- and gamma-tubulin. EMBO J. 17:952–960.
9. Ghaemmaghami, S., W. K. Huh, K. Bower, R. W. Howson, A. Bets, N. Dephoure, E. K. O’Shea, and J. S. Weissman. 2003. Global analysis of protein expression in yeast. Nature 425:737–741.
10. Hermann, G. J., J. E. King, and J. M. Shaw. 1997. The yeast gene, MDM20, is necessary for mitochondrial inheritance and organization of the actin cytoskeleton. J. Cell Biol. 137:141–153.
11. Huh, W. K., J. V. Falvo, L. C. Gerke, A. S. Carroll, R. W. Howson, J. S. Weissman, and E. K. O’Shea. 2003. Global analysis of protein localization in budding yeast. Nature 425:916–920.
12. Jansen, R., and M. Gerstman. 2000. Analysis of the yeast transcriptome with structural and functional categories: characterizing highly expressed proteins. Nucleic Acids Res. 28:1481–1488.
13. Kaufman, P. D., R. Kobayashi, and B. Stillman. 1997. Ultraviolet radiation sensitivity and reduction of telomeric silencing in Saccharomyces cerevisiae cells lacking chromatin assembly factor-I. Genes Dev. 11:345–357.
14. Kimura, Y., Y. Saeki, H. Yokosawa, B. Polevoda, F. Sherman, and H. Hirano. 2003. N-terminal modifications of the 19S regulatory particle subunits of the proteosome. Arch. Biochem. Biophys. 409:341–348.
15. Krogan, N. J., M. C. Kegoh, N. Datta, C. Sawa, O. W. Ryan, H. Ding, R. A. Jaw, J. Pootoolal, A. Tong, V. Canadian, D. P. Richards, X. Wu, A. Emili, T. R. Hughes, S. Buratowski, and J. F. Greenblatt. 2003. A Sm2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol. Cell 12:1565–1576.
16. Lee, F. J., L. W. Lin, and J. A. Smith. 1989. N alpha-acetyltransferase deficiency alters protein synthesis in Saccharomyces cerevisiae. FEBS Lett. 256:139–142.
17. Mullen, J. R., P. S. Kayne, R. P. Moerschell, S. Tsumasawa, M. Gribkov, M. Colavito-Shepanski, M. Grunstein, F. Sherman, and R. Stierling. 1989. Identification and characterization of genes and mutants for an N-terminal acetyltransferase from yeast. EMBO J. 8:2067–2075.
18. Neuwald, A. F., and D. Landsman. 1997. GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. Trends Biochem. Sci. 22:154–155.
19. Persson, B., R. Flinta, G. von Heijne, and H. Jornvall. 1985. Structures of N-termiially acetylated proteins. Eur. J. Biochem. 152:523–527.
20. Polevoda, B., T. S. Cardillo, T. C. Doyle, G. S. Bedi, and F. Sherman. 2003.
Nat3p and Mdm20p are required for function of yeast NatB Nalpha-terminal acetyltransferase and of actin and tropomyosin. J. Biol. Chem. 278:30686–30697.

21. Polevoda, B., J. Norbeck, H. Takakura, A. Blomberg, and F. Sherman. 1999. Identification and specificities of N-terminal acetyltransferases from Saccharomyces cerevisiae. EMBO J. 18:6155–6168.

22. Polevoda, B., and F. Sherman. 2001. NatC Nalpha-terminal acetyltransferase of yeast contains three subunits, Mak3p, Mak10p, and Mak31p. J. Biol. Chem. 276:20154–20159.

23. Polevoda, B., and F. Sherman. 2003. N-terminal acetyltransferases and sequence requirements for N-terminal acetylation of eukaryotic proteins. J. Mol. Biol. 325:595–622.

24. Rigaut, G., A. Shevchenko, B. Rutz, M. Wilm, M. Mann, and B. Seraphin. 1999. A generic protein purification method for protein complex characterization and proteome exploration. Nat. Biotechnol. 17:1030–1032.

25. Setty, S. R., T. I. Strochlic, A. H. Tong, C. Boone, and C. G. Burd. 2004. Golgi targeting of ARF-like GTPase Arl3p requires its Nalpha-acetylation and the integral membrane protein Sys1p. Nat. Cell Biol. 6:414–419.

26. Sharp, J. A., D. C. Krawitz, K. A. Gardner, C. A. Fox, and P. D. Kaufman. 2003. The budding yeast silencing protein Sir1 is a functional component of centromeric chromatin. Genes Dev. 17:2356–2361.

27. Siegers, K., T. Waldmann, M. R. Leroux, K. Grein, A. Shevchenko, E. Schiebel, and F. U. Hartl. 1999. Compartmentation of protein folding in vivo: sequestration of non-native polypeptide by the chaperonin-GimC system. EMBO J. 18:75–84.

28. Singer, J. M., G. J. Hermann, and J. M. Shaw. 2000. Suppressors of mdm20 in yeast identify new alleles of ACT1 and TPM1 predicted to enhance actin-tropomyosin interactions. Genetics 156:523–534.

29. Singer, J. M., and J. M. Shaw. 2003. Mdm20 protein functions with Nat3 protein to acetylate Tpm1 protein and regulate tropomyosin-actin interactions in budding yeast. Proc. Natl. Acad. Sci. USA 100:7644–7649.

30. Song, O. K., X. Wang, J. H. Waterborg, and R. Sternglanz. 2003. An Nalpha-acetyltransferase responsible for acetylation of the N-terminal residues of histones H4 and H2A. J. Biol. Chem. 278:38109–38112.

31. Wach, A. 1996. PCR-synthesis of marker cassettes with long flanking homology regions for gene disruptions in S. cerevisiae. Yeast 12:259–265.

32. Walker, J.-P. 1963. The NH2-terminal residues of the proteins from cell-free extracts of E. coli. J. Mol. Biol. 7:483–496.

33. Wang, X., J. J. Connelly, C. L. Wang, and R. Sternglanz. 2004. Importance of the Sir3 N terminus and its acetylation for yeast transcriptional silencing. Genetics 168:547–551.

34. Warringer, J., and A. Blomberg. 2003. Automated screening in environmental arrays allows analysis of quantitative phenotypic profiles in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 100:15724–15729.