NatC N\(^\alpha\)-Terminal Acetyltransferase of Yeast Contains Three Subunits, Mak3p, Mak10p, and Mak31p*

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The yeast Saccharomyces cerevisiae contains three types of N\(^\alpha\)-terminal acetyltransferases, NatA, NatB, and NatC, with each having a different catalytic subunit, Ard1p, Nat3p, and Mak3p, respectively, and each acetylating different sets of proteins with different N\(^\alpha\)-terminal regions. We show that the NatC N\(^\alpha\)-terminal acetyltransferases contains Mak10p and Mak31p subunits, in addition to Mak3p, and that all three subunits are associated with each other to form the active complex. Genetic deletion of any one of the three subunits results in identical abnormal phenotypes, including the lack of acetylation of a NatC substrate in vivo, diminished growth at 37 °C on media containing nonfermentable carbon sources, and the lack of maintenance or assembly of the L-A dsRNA viral particle.

The two cotranslational processes, cleavage of N-terminal methionine residues and N-terminal acetylation, are the most common modifications, occurring on the vast majority of eukaryotic proteins. Methionine residues at the N termini are cleaved from nascent chains of most proteins, and subsequently N-terminal acetylation occurs on certain of the proteins, either those containing or lacking the methionine residue (reviewed in Ref. 1). As summarized in Table I, Saccharomyces cerevisiae contains three types of N-terminal acetyltransferases, NatA, NatB, and NatC, with each having a different catalytic subunit, Ard1p, Nat3p, and Mak3p, respectively, and each acetylating different sets of proteins with different N-terminal regions (1, 2). In addition, the NatA N-terminal acetyltransferase contains another subunit, Nat1p, which associates with the catalytic subunit, Ard1p, and which is required for activity (3, 4).

The substrate specificities for each of NatA, NatB, and NatC were deduced by considering the lack of acetylation of the following groups of protein in mutants containing one or another of the ard1-D, nat1-D, nat3-D, or mak3-D deletions: mutationally altered iso-1-cytochromes c (iso-1) (2, 5, 6), mutationally altered \(\beta\)-galactosidases (7), abundant proteins (2, 8, 9), ribosomal proteins (10), and 20 S proteasome subunits (11).

Subclasses of proteins with Ser, Ala, Gly or Thr termini are not acetylated in ard1-D or nat1-D mutants (NatA substrates); proteins with Met-Glu or Met-Asp termini and subclasses of proteins with Met-Asn termini are not acetylated in nat3-D mutants (NatB substrates); and subclasses of proteins with Met-Ile, Met-Leu, Met-Trp, or Met-Phe termini are not acetylated in mak3-D mutants (NatC substrates). We wish to emphasize that acetylation occurs only on subsets of these proteins, and the presence of the specified termini does not guarantee N-terminal acetylation. In addition, a special subclass of NatA substrates with Ser-Glu, Ser-Asp, Ala-Glu, or Gly-Glu termini, previously designated NatD substrates and now designated NatA' substrates, may not be completely acetylated in nat3-D and mak3-D mutants (1, 2, 11).

Mak3\(^c\), encoding the catalytic subunit of NatC, is required for the N-terminal acetylation of the killer viral major coat protein, Gag, with a mature Ac-Met-Leu-Arg-Phe terminus. Mak3 was first identified from mak3-D-deficient mutants that did not assemble or maintain the L-A dsRNA viral particle (12). Mak3-D-deficient mutants also have reduced growth on media containing nonfermentable carbon sources as the sole source of energy, such as glycerol or ethanol. Tercero et al. (7) suggest that the diminished growth of mak3-D strains on glycerol medium is because of lack of N-terminal acetylation of the mitochondrial proteins Kdg1p (\(\alpha\)-ketoglutarate dehydrogenase), Fum1p (fumarate dehydratase), and Mrp1p (a mitochondrial ribosomal protein), that all contain Met-Leu-Arg-Phe terminal, similar to the L-A Gag protein. NatC substrates are rare, and none were encountered among 55 abundant proteins (2) or among 68 ribosomal proteins (10), but two were uncovered, Pup2p and Pre6p, among 14 20 S proteasome subunits, both containing Met-Phe N termini (11).

There are 29 MAK genes defined on the basis of their requirement for stable propagation of M\(_1\) dsRNA (13). Their modes of action are diverse, with the bulk of the MAK genes involved in the 60 S ribosomal subunit biogenesis. Mak3p, Mak10p, and Mak31p co-purify, suggesting that they constitute three subunits of a complex (14). Moreover, protein-protein interactions between Mak3p and Mak10p, as well as between Mak31p and Mak10, were detected in a two-hybrid screen (15). Significantly, of all of the MAK genes, only MAK3, MAK10, and MAK31 were found necessary for L-A virus propagation (16–18). Furthermore, similar to mak3 mutants, mak10 mutants grew slowly on media containing nonfermentable carbon sources (19). Although the biological function of the MAK31 gene was not previously known, Seraphin (20) suggested that Mak31p is an Sm-like protein based on protein sequence similarity. Sm-like proteins are small nuclear ribonucleoproteins (snRNPs) found associated with U1, U2, and U5 snRNAs, as well as with U4/U6 double snRNPs and the U4/U6/U5 triple snRNP. snRNPs are involved in various functions including pre-mRNA splicing, histone mRNA 3’-formation,
tRNA processing, rRNA maturation, and telomeric DNA synthesis (21). However, Mak31p is more divergent compared with other yeast Sm-like proteins, and it is the only member lacking a glycine or cysteine at position 107 as numbered according to the alignment of Sm domains. Furthermore, Mak31p did not precipitate any of the tested RNAs (20).

We have investigated the requirements of the Mak3p, Mak10p, and Mak31p subunits for acetylation with the yeast iso-1 system. Because the N-terminal region of iso-1 is dispensable for biosynthesis, function, and mitochondrial import (22, 23), N-terminal processing can be investigated freely with essentially any alteration. In fact, altered forms of iso-1 proved to be ideally suited for investigating the specificity of N-terminal methionine cleavage and N-terminal acetylation (2, 5, 6). In addition, because the mass of iso-1 is ~12.5 kDa and because mass spectrometry can be used conveniently to determine molecules less than 30 kDa with an accuracy of ~1 Da, we have used this method to determine acetylation of mutant forms of iso-1.

In this study, we have demonstrated that each of the three subunits, Mak3p, Mak10p, and Mak31p, is absolutely required for N-terminal acetylation of a NatC type of substrate in vivo. Deletion of any of the corresponding genes prevented acetylation of a NatC type of altered iso-1 but not of a NatA or NatB types of iso-1. We have also shown that all three deletion strains showed similar phenotypes, including slower growth on nonfermentable carbon sources at elevated temperatures. Thus, all three subunits (Mak3p, Mak10p, and Mak31p) of the complex are required for NatC activity.

MATERIALS AND METHODS

Genetic Nomenclature—Using standard genetic nomenclature, MAK3, for example, designates the normal wild-type allele; mak3Δ designates the deletion or disruption of the gene; and Mak3p designates the protein encoded by MAK3. CYC1–853, CYC1–987, etc., designate

### Table I
The three types of N-terminal acetyltransferases

| Type   | NatA | NatB | NatC |
|--------|------|------|------|
| Catalytic subunit | Ard1p | Nat3p | Mak3p |
| Other subunits | Ser | Met-Glu | Met-Ile |
| | Ala | Met-Asp | Met-Leu |
| | Gly | Met-Asn | Met-Trp |
| | Thr | Met-Met | Met-Phe |

*Acetylation occurs only on subclasses of proteins containing the indicated termini, except for Met-Glu and Met-Asp termini, which are apparently always acetylated.

### Table II
Yeast strains

| Strain | Genotype |
|--------|----------|
| B-7528  | MATa cycl7–67 ura3–52 lys5–10 cycl1–31 |
| B-12479 | MATa cycl7–67 ura3–52 lys5–10 CYC1–1383 |
| B-13393 | MATa cycl7–67 ura3–52 lys5–10 CYC1–1383 nat1Δ::URA3 |
| B-13739 | MATa cycl7–67 ura3–52 lys5–10 CYC1–1383 mak3Δ::URA3 |
| B-7687  | MATa cycl7–67 ura3–52 lys5–10 CYC1–853 |
| B-7727  | MATa cycl7–67 ura3–52 lys5–10 CYC1–853 nat1Δ::URA3 |
| B-9072  | MATa cycl7–67 ura3–52 lys5–10 CYC1–853 mak3Δ::URA3 |
| B-11863 | MATa cycl7–67 ura3–52 lys5–10 CYC1–853 nat3Δ::kanMX4 |
| B-13274 | MATa cycl7–67 ura3–52 lys5–10 CYC1–853 mak31Δ::kanMX4 |
| B-8462  | MATa cycl7–67 ura3–52 lys5–10 CYC1–1162 |
| B-9022  | MATa cycl7–67 ura3–52 lys5–10 CYC1–1162 mak3Δ::URA3 |
| B-9024  | MATa cycl7–67 ura3–52 lys5–10 CYC1–1162 nat1Δ::URA3 |
| B-11865 | MATa cycl7–67 ura3–52 lys5–10 CYC1–1162 nat3Δ::kanMX4 |
| B-12333 | MATa cycl7–67 ura3–52 lys5–10 CYC1–1162 mak10Δ::kanMX4 |
| B-13275 | MATa cycl7–67 ura3–52 lys5–10 CYC1–1162 mak31Δ::kanMX4 |
| B-10645 | MATa cycl7–67 ura3–52 lys5–10 CYC1–987 |
| B-10672 | MATa cycl7–67 ura3–52 lys5–10 CYC1–987 mak3Δ::URA3 |
| B-10689 | MATa cycl7–67 ura3–52 lys5–10 CYC1–987 nat1Δ::URA3 |
| B-12045 | MATa cycl7–67 ura3–52 lys5–10 CYC1–987 nat3Δ::kanMX4 |
| B-12322 | MATa cycl7–67 ura3–52 lys5–10 CYC1–987 mak10Δ::kanMX4 |
| B-13276 | MATa cycl7–67 ura3–52 lys5–10 CYC1–987 mak31Δ::kanMX4 |
| B-6925  | MATa cycl7–67 trp2 can1 lys2 cycl1–115 |

### Table III
Oligonucleotides used in the construction and testing of disrupted genes

The position of the first nucleotide is presented, where A of the ATG initiation codon is assigned position 1. The underlined sequences correspond to segments in the plasmid pFA6-kanMX4. ORF, open reading frame.

| ORF | Oligo | Sequence (5' → 3') |
|-----|-------|-------------------|
| NAT3 | Oligo 1 | (-70) GCACACACATACAGCCTTGCGGCGCACTTGTTTGCCCTGAGAACAGCTGAGCTTCG |
|     | Oligo 2 | (+114) TGAATAGCAGAGGTTCATTATTATGTTGTGAGATAGGAGCAAGCCGCAGTGTGAGATCG |
|     | Oligo 3 | (-142) TCTCAATAGCAGAGGCTTCACATAC |
|     | Oligo 4 | (+906) TATAATCACATGATGACATAC |
| NAT1 | Oligo 5 | (-41) GACAAATACCTTACGAGGAGGC |
|     | Oligo 6 | (+2741) GAAAGAAGTGTCCGGCAATGGTCC |
| MAK3 | Oligo 7 | (-143) CCGGATGCAAAGGCA |
|     | Oligo 8 | (+679) ACTTCTTTATATCCCTCC |
| MAK31 | Oligo 9 | (-51) AACGTCGCTATGTGTAAGACAAACCTTTGATAATAGCCTGTTAGGCTTCCAGAACAGCTGAGGCTTCG |
|     | Oligo 10 | (+2255) TGGGGGCTTATATAGGTAATATGGATAGGTAATATGGGGGTCATAGGCTGAGATGAGTC |
|     | Oligo 11 | (-133) GAGATGGAGTAAAATTTCCAG |
|     | Oligo 12 | (+2318) TGCGAATGACATGAGGCT |
| MAK31 | Oligo 13 | (-53) TTGATAGGGGAGAGATAGCTCCTCCAGAAACCTTTAGTGAAGATGGGCAGCACAGCCTGAGGCTTCG |
|     | Oligo 14 | (+311) TGGCAAGACATTGTCCTGGAGAAACATAGTAGAACCATGATACAGGAGGCTGAGATGAGTC |
|     | Oligo 15 | (-100) CGCGAAAGTGTCCATGAGGCT |
|     | Oligo 16 | (+362) AGTTCCTGAGCTATATTCG |
genes encoding different mutant forms of functional iso-1, whereas cyc1–3 and cyc1–115 designate alleles lacking or encoding nonfunctional forms of iso-1. The cyc7–67 allele denotes a partial deletion of the CYC7 gene that results in complete deficiency of iso-2-cytochrome c.

**Yeast Strains and Media**—Unless stated otherwise, yeast was grown at 30 °C in YPD or YPG medium or SD medium containing appropriate supplements (24). The strains used in this study are listed in Table II. The analysis of N-terminal acetylation was carried out with a series of isogenic strains originally derived from B-7528 (cyc1–31 MATa cyc7–67 ura3–52 lys5–10). The series of strains used for producing altered iso-1

![Genes encoding different mutant forms of functional iso-1, whereas cyc1–3 and cyc1–115 designate alleles lacking or encoding nonfunctional forms of iso-1. The cyc7–67 allele denotes a partial deletion of the CYC7 gene that results in complete deficiency of iso-2-cytochrome c.](image)

**TABLE IV**

| Substrate typea | Alleleb | Sequence | % Acetylation | % Iso-1c Normal | mak3-Δ | mak10-Δ | mak31-Δ | nat1-Δ | nat3-Δ |
|-----------------|---------|----------|---------------|----------------|--------|--------|--------|--------|--------|
| NatA CYC1–1383 | Ac (Met)Ser-Glu-Ile-Thr-Ala-ATA ATG TCT GAA ATC AGG GCC | 100 | 100 | 0 |
| NatB CYC1–853  | Ac-Met-Glu-Phe-Leu-Ala-ATA ATG GAA TTC TGG GCC | 80 | 100 (100) | 100 (100) | 0 |
| NatC CYC1–1162 | Ac-Met-Ile-Arg-Leu-Lys-Ala-ATA ATG ATG AGA TGG AAG GCC | 30 | 100 | 0 | 0 | 0 | 100 | 100 |
| NatA’ CYC1–987 | (Met)·Ser-Glu-Phe-Leu-Ala-ATA ATG TCT GAA TTI TGG GCC | 25 | 100 | ~95 | ~95 | ~95 | (0) | ~90 |

a The substrate type designates the substrates that are exemplified by the N-terminal sequence of the altered iso-1 shown in column 3 (see Table I).
b The results were obtained with the strains listed in Table II.
c Designates the level (%) of iso-1-cytochrome c in comparison with the isogenic CYC1+ strain.

**TABLE V**

| Substrate type | CYC1 allele | Normal | mak3-Δ | mak10-Δ | mak31-Δ | nat1-Δ | nat3-Δ |
|----------------|-------------|--------|--------|--------|--------|--------|--------|
| NatA CYC1–1383 | Strain B-12479 | B-13739 | B-13393 | B-13393 | B-13393 | B-13393 | B-13393 |
| NatB CYC1–853  | Mass (Da) B-7687 | B-9072 | B-13274 | B-7223 | B-13274 | B-13274 | B-13274 |
| NatC CYC1–1162 | Observed 12,671 | 12,672 | 12,638 | 12,638 | 12,642 | 12,642 | 12,642 |
| NatA’ CYC1–987 | Expected 12,684 | 12,684 | 12,684 | 12,684 | 12,684 | 12,684 | 12,684 |
| NatB CYC1–853  | % Acetylation 100 | 100 | 0 | 0 | 0 | 0 | 0 |
| NatC CYC1–1162 | Strain B-8462 | B-9022 | B-12333 | B-13275 | B-9024 | B-13275 | B-9024 |
| NatA’ CYC1–987 | Mass (Da) B-8462 | B-9022 | B-12333 | B-13275 | B-9024 | B-13275 | B-9024 |
| NatB CYC1–853  | Observed 12,884 | 12,892 | 12,892 | 12,892 | 12,892 | 12,892 | 12,892 |
| NatC CYC1–1162 | Expected 12,892 | 12,892 | 12,892 | 12,892 | 12,892 | 12,892 | 12,892 |
| NatA’ CYC1–987 | % Acetylation 100 | 100 | 0 | 0 | 0 | 0 | 0 |
| NatB CYC1–853  | Strain B-10645 | B-10672 | B-12332 | B-13276 | B-10689 | B-13276 | B-10689 |
| NatC CYC1–1162 | Mass (Da) B-10645 | B-10672 | B-12332 | B-13276 | B-10689 | B-13276 | B-10689 |
| NatA’ CYC1–987 | Observed 12,718 | 12,718 | 12,718 | 12,718 | 12,718 | 12,718 | 12,718 |
| NatB CYC1–853  | Expected 12,730 | 12,730 | 12,730 | 12,730 | 12,730 | 12,730 | 12,730 |
| NatC CYC1–1162 | % Acetylation 100 | 100 | 0 | 0 | 0 | 0 | 0 |
| NatA’ CYC1–987 | Strain B-10645 | B-10672 | B-12332 | B-13276 | B-10689 | B-13276 | B-10689 |
| NatB CYC1–853  | Mass (Da) B-10645 | B-10672 | B-12332 | B-13276 | B-10689 | B-13276 | B-10689 |
| NatC CYC1–1162 | Observed 12,718 | 12,718 | 12,718 | 12,718 | 12,718 | 12,718 | 12,718 |
| NatA’ CYC1–987 | Expected 12,730 | 12,730 | 12,730 | 12,730 | 12,730 | 12,730 | 12,730 |
| NatB CYC1–853  | % Acetylation 100 | ~95 | ~95 | ~95 | ~95 | ~95 | ~95 |
| NatC CYC1–1162 | Strain B-10645 | B-10672 | B-12332 | B-13276 | B-10689 | B-13276 | B-10689 |
| NatA’ CYC1–987 | Mass (Da) B-10645 | B-10672 | B-12332 | B-13276 | B-10689 | B-13276 | B-10689 |
| NatB CYC1–853  | Observed 12,718 | 12,718 | 12,718 | 12,718 | 12,718 | 12,718 | 12,718 |
| NatC CYC1–1162 | Expected 12,730 | 12,730 | 12,730 | 12,730 | 12,730 | 12,730 | 12,730 |
| NatA’ CYC1–987 | % Acetylation 100 | ~95 | ~95 | ~95 | ~95 | ~95 | ~95 |
were constructed first by using synthetic oligonucleotides to generate CYC1 mutations encoding the desired iso-1 and second by separately disrupting each of the MAK3, MAK10, MAK31, NAT1, or NAT3 genes (see below). Strains are designated as “normal” if they contain the full complement of normal NAT genes.

**Mating Efficiencies**—Quantitative matings were determined by plat- ing serial dilutions of logarithmically growing yeast cells onto SD plates containing a lawn of the tester mating strain, B-6925, and determining the frequencies of prototrophic diploid colonies arising after incubation for 3 days. Dilutions of the haploid strains were also plated on YPD plates to determine total number of cell. Mating efficiencies were ex- pressed as the ratio of the number of diploid colonies to the number of haploid cells plated on the lawn of the tester strain.

**Construction of Iso-1 Mutants**—Strains with altered forms of iso-1 were conveniently produced by transforming yeast directly with syn- thetic oligonucleotides as described previously by Yamamoto et al. (25), using the cycl-31 mutant B-7528 (Table II) and 50 μg of the oligonu-}

**RESULTS AND DISCUSSION**

Phenotypes of mak Deletion Mutants—It has been previously reported that mak3-Δ (31) and mak10-Δ (19) mutants have diminished growth on medium with glycerol or ethanol as a carbon source. These phenotypes were confirmed and others were investigated in this study using the following isogenic series of strains that were prepared from strain B-7528 by making null deletions of MAK3, MAK10, and MAK31 genes by disrupter plasmids (see “Materials and Methods”) or PCR-based disrupters (see Table III for corresponding oligonucleo- tidies); normal (B-8462), mak3-Δ (B-9072), mak10-Δ (B-12333), and mak31-Δ (B-12375). As shown in Fig. 1, mak3-Δ, mak10-Δ, and mak31-Δ mutants grew poorly on YPG medium at 37 °C, although growth on YPD medium at 37 °C was normal, thus display- ing the Nfs- phenotype (diminished growth on media containing nonfermentable substrates and energy source). Similar reduced growth for all of the deletion mutants was detected on YPE (ethanol), YPGal (galactose), and YPRaf (raffinose) media (not shown). (Because the normal strain, B-8462 is Gal- and Raf-/-, the diminished growth on YPGal and YPRaf media is a secondary consequence of the Nfs- phenotype; ρ- derivatives of B-8462 also did not grow on...
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Fig. 3. Amino acid sequence alignments of Mak3p (A) and Mak31p (B) orthologs. Protein sequences were aligned using Multalin, version 5.4.1 (35). Highly conserved residues are highlighted in black, and moderately conserved residues are highlighted in gray. Consensus symbols are: !, any one of IV; $, any one of LM; #, any one of FY; %, any one of NDQE. The GenBank sequences (GenBank™ and PIR) are as follows: A. thaliana, AT102145, AT1AAG-51452 (plant); C. elegans, CeAB65889, CeAA235212 (invertebrate); D. melanogaster, DmA617683, DmA417656 (invertebrate); H. sapiens, HsAB14397, HsAA62222 (mammal); M. musculus, MmBA627439 (mammal); M. sativa, MsCA414975 (plant); S. cerevisiae, Mak3p, Mak31p (fungus); S. pombe, SpT39482, SpT417178 (fungus); T. aquaticum, TaCAC11206 (archaea).

YPGal and grew poorly on YPra media, especially at 37 °C. Also, the mating frequencies of mak3-Δ (B-9022), mak10-Δ (B-12333), and mak31-Δ (B-12375) mutants were the same or similar to the mating efficiency of normal strain B-8462 (not shown), a result that is in contrast to the results with the ard1, nat1, and nat3 mutants, which have reduced mating efficiencies (2). In addition, iso-1 levels in the mak3-Δ, mak10-Δ, and mak31-Δ deletion mutants did not differ significantly from the corresponding normal MAK+ strains, as estimated by low-temperature spectroscopic examination of intact cells (Table IV). It is noteworthy that the mak3-Δ (B-9022), mak10-Δ (B-12333), and mak31-Δ (B-12375) mutant strains contained the normal complement of all of the cytochromes when grown on YPD medium at 37 °C. Thus, the lack of growth at 37 °C on media with nonfermentable carbon sources cannot be attributed to a cytochrome deficiency.

Lack of Acetylation of Altered Iso-1 in Deletion Mutants—For this study, we chose four different CYC1 alleles with corresponding altered iso-1 N termini, each representing one class of NAT substrates (Table IV). Iso-1 from the normal strain and from each of the mak3-Δ, mak10-Δ, mak31-Δ, nat1-Δ, and nat3-Δ mutants were subjected to mass spectrometric analysis. Peaks corresponding to iso-1 were observed at masses between 12.5 and 13 kDa depending on the altered N-terminal sequence. The iso-1 were identified by the masses determined from the spectra, the masses deduced from the gene sequences, and knowledge of previously established modifications. The results of MALDI-TOF determinations of molecular masses for altered iso-1 are presented in Table V. Examples of some spectra are shown in Fig. 2. The lack of protein acetylation leads to a diminished molecular mass of ~42 Da, which corresponds to the mass of the acetyl group. In general, the samples were either completely or almost completely acetylated or were completely unacetylated, except for a NatA’ substrate from CYC1-987 strains having one or another of the mak3-Δ, mak10-Δ, mak31-Δ, and nat3-Δ deletions in which the iso-1 contained ~5–10% of the unacetylated form.

Overall, the analysis of iso-1 acetylation from the complete sets of isogenic strains gave rise to expected results (Table IV and V). The decrease of the molecular mass of Ser-Glu-Ile (CYC1–1383) iso-1 by ~42 Da, indicative of the lack of acetylation, was observed in B-13393 (nat1-Δ mutant) but not in B-13739 (mak3-Δ mutant). The Met-Glu-Phe (CYC1–853) iso-1 was not acetylated in B-11863 (nat3-Δ mutant) but remained acetylated in the B-7723 (nat1-Δ), B-9072 (mak3-Δ), and B-13274 (mak31-Δ) mutants. The Met-Ile-Arg (CYC1–1162) iso-1 was not acetylated in the B-9022 (mak3-Δ) mutant but was acetylated in the B-9024 (nat1-Δ) and B-11865 (nat3-Δ) mutants. The Ser-Glu-Phe (CYC1–987) iso-1 was acetylated in the B-12332 (mak10-Δ) and B-13275 (mak31-Δ) mutants but not in B-10689 (nat1-Δ). Also, CYC1–987 iso-1 from B-10672 (mak3-Δ) and B-12045 (nat3-Δ) were partially acetylated (Fig. 2). All of these results could be predicted from the previously identified NAT substrates (2). Importantly, acetylation was not detected in altered iso-1 Met-Ile-Arg (CYC1–1162) from B-12333 (mak10-Δ) and B-13275 (mak31-Δ) mutants. Thus, the presence of the functional Mak3p, Mak10p, and Mak31p is absolutely required for acetylation of NatC-type substrates in vivo.

Orthologs of Mak3p, Mak10p, and Mak31p—One important question concerns the N-terminal acetyltransferases and the nature of N-terminal acetylation in other eukaryotes. Morschell et al. (6) point out the similarity in the pattern of N-terminal acetylation of proteins from higher eukaryotes and S. cerevisiae, suggesting that the same systems may operate in all eukaryotes. In addition, Polevoda, Sherman, and colleagues (2) point out the existence of orthologous genes encoding the catalytic subunits of the three N-terminal acetyltransferases, also indicative of the same or similar N-terminal acetyltransferases operating in higher eukaryotes. Species containing orthologs of the yeast Ard1p and Nat3p include Caenorhabditis elegans, Drosophila melanogaster, Homo sapiens, as well as others (2).

As shown in Fig. 3A, BLAST (basic local alignment search tool program) (33) comparisons revealed that species containing orthologs of the yeast Mak3p include Schizosaccharomyces pombe, C. elegans, D. melanogaster, Mus musculus, Arabidopsis thaliana, and H. sapiens. Also proteins with high similarity to Mak3p are present in Archaeoglobus fulgidus, Aeropyrum pernix, Campylobacter coli, Methanobacterium thermoautotrophicum, Methanococcus jannaschii, Pyrococcus abyssi, Pyrococcus horikoshii, Sulfolobus solfataricus, Ureaplasma urealyticum, and Escherichia coli. However, it is doubtful that the similar proteins in prokaryotes are N-terminal acetyltransferases that act co-translationally on a wide range of proteins but are rather more similar, for example, to E. coli RimIp that acetylates the N terminus of ribosomal protein S18 (34).

BLAST comparisons also revealed orthologous genes encoding snRNP Sm-like proteins, similar to Mak31p, in S. pombe,
Medicago sativa, Thermoplasma acidophilum, C. elegans, D. melanogaster, A. thaliana, and H. sapiens, with 35–55% amino acid sequence similarity spanning almost the entire protein (Fig. 3B). Although it is unclear whether these proteins have Sm or Mak31p functions, the Gly or Cys residues at position 107 conserved in Sm proteins are not found in these proteins.

Lee and Wickner (19) have previously pointed out the sequence similarity of Mak10p to the variable regions of the T cell proteins. 107 conserved in Sm proteins are not found in these defined proteins are not N-terminal acetyltransferases, and the similarities may reflect the presence of one or two hypothetical transmembrane domains in Mak10p. Nevertheless, it is unknown whether Mak10p is a Mak3p-Mak10p-Mak31p complex and that each of the subunits is required for acetylating NatC-type substrates in vivo, including the killer viral major coat protein, Gag, and an unidentified component responsible for normal utilization of nonfermentable substrates.

Conclusions—We conclude that the NatC N-terminal acetyltransferase is a Mak3p-Mak10p-Mak31p complex and that each of the subunits is required for acetylating NatC-type substrates in vivo, including the killer viral major coat protein, Gag, and an unidentified component responsible for normal utilization of nonfermentable substrates.

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