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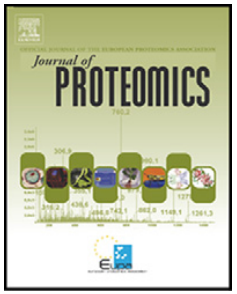

N^{α}-Acetylation of yeast ribosomal proteins and its effect on protein synthesis

Masahiro Kamita[a], *Yayoi Kimura*[a], *Yoko Ino*[a], *Roza M. Kamp*[b], *Bogdan Polevoda*[c], *Fred Sherman*[c], *Hisashi Hirano*[a,*]

[a]*Graduate School of Nanobioscience, Yokohama City University, Suehiro 1-7-29, Tsurumi, Yokohama 230-0045, Japan*
[b]*University of Applied Science and Technology, 13347 Berlin, Germany*
[c]*Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, NY 14642, USA*

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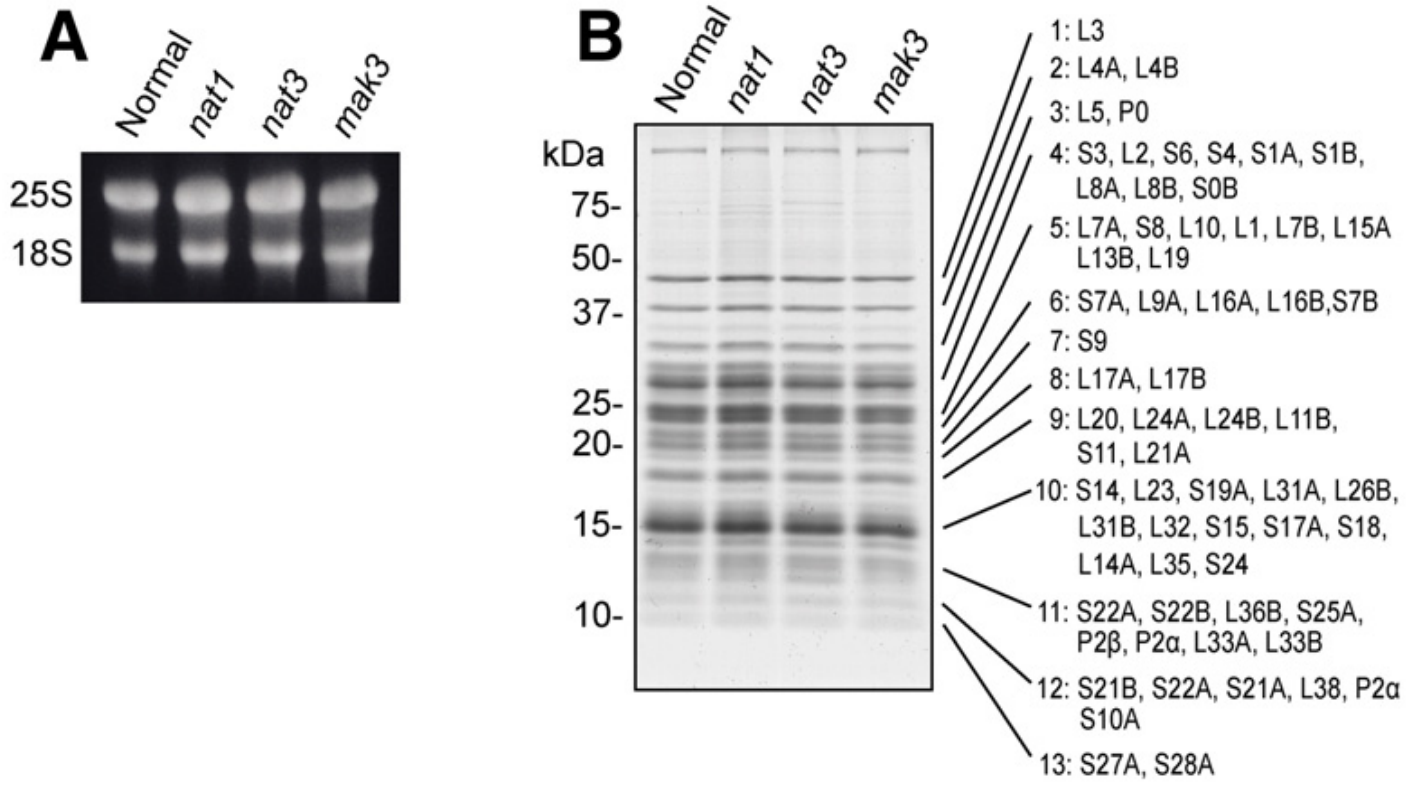
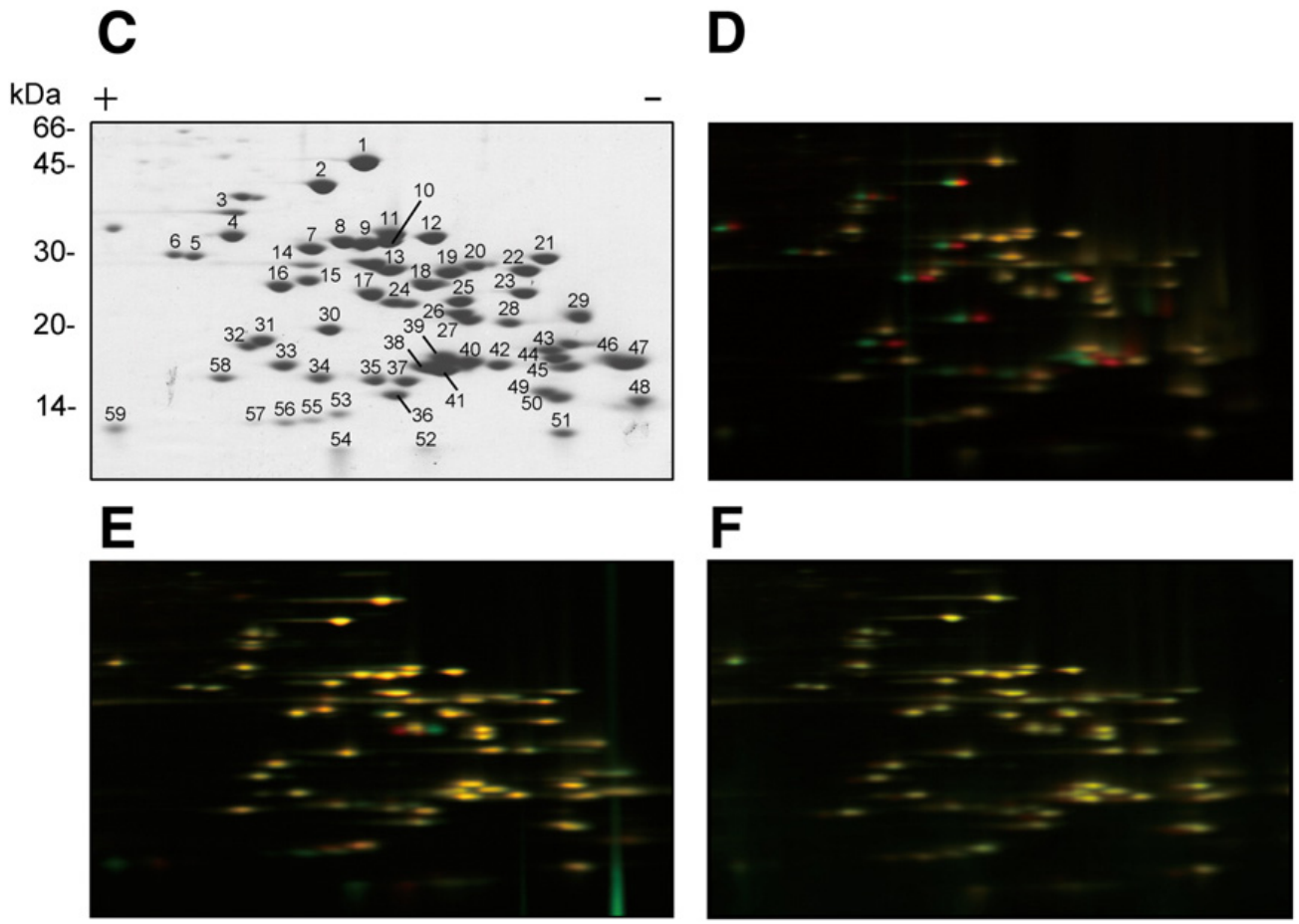


Fig. 1 – Analysis of rRNAs and N^{α}-acetylated ribosomal proteins by 2D-DIGE. (A) Purified rRNA from yeast 80S ribosomes was separated by agarose gel electrophoresis and stained with ethidium bromide. (B) Purified ribosomal proteins from yeast 80S ribosomes were separated by SDS-PAGE and stained with CBB R-250. The details of MS data using an ESI-LIT-TOF MS was shown in Supplementary Table 1. (C) Purified ribosomal proteins from yeast 80S ribosomes were separated by 2-DE using acid-



shown in Supplementary Table 1. (C) Purified ribosomal proteins from yeast 80S ribosomes were separated by 2-DE using acid-urea electrophoresis in the first dimension and SDS-PAGE in the second dimension and stained with CBB R-250. Protein spots identified by an ESI-Q-TOF-MS are numbered and their details were indicated in Supplementary Table 2. (D–F) Identification of the N^{α}-acetylated ribosomal proteins from the *nat1*, *nat3*, and *mak3* mutants, respectively. Equal amounts of purified ribosomal proteins from the normal strain and the NAT mutant were labeled with Cy3 and Cy5 respectively. The Cy-labeled ribosomal proteins from the two strains were separated on the same 2-DE gel and the Cy3- and Cy5-images were compared. The N^{α}-acetylated ribosomal proteins are shown in Table 1.

Table 1 – N^{α}-Acetylation of ribosomal proteins.

Subunit	Protein	MW (kDa) [a]	pI [a]	2-DE [b]	TOF-MS [c]	DIGE	NAT
60S	P0	33.7	4.6	–	–	–	–
	P1 A/B	10.9/10.6	3.6/3.7	–	●/–	–	–
	P2 A/B	10.7/11.1	3.8/3.9	–	○/○	–	–
	L1 A/B [d]	24.5/24.5	9.7/9.7	–	●/●	●/●	NatA
	L2 A/B [d]	27.4/27.4	11.1/11.1	○/–	–	○/○	–
	L3	43.7	10.3	○	–	○	–
	L4 A/B	39.0/39.0	10.6/10.6	●/–	–	●/●	NatA
	L5	33.7	6.4	–	–	○	–
	L6 A/B	19.9/20.0	10.1/10.1	–	●/–	○/○	–
	L7 A/B	27.6/27.7	10.2/10.2	○/–	○/–	○/○	–
	L8 A/B	28.1/28.1	10.0/10.0	○/–	○/–	○/○	–
	L9 A/B	21.6/21.7	9.7/10.5	–	–	○/–	–
	L10	25.4	10.8	–	○	–	–
	L11 A/B	19.7/19.7	9.9/9.9	●	●/●	●/●	NatA
	L12 A/B [d]	17.8/17.8	9.4/9.4	–	–	○/○	–
	L13 A/B	22.5/22.5	11.2/11.7	–	–	○/–	–
	L14 A/B	15.2/15.2	10.4/11.6	–	●/–	●/–	NatA
	L15 A/B [d]	24.4/24.4	11.4/12.0	○/–	–	○/–	–
	L16 A/B	22.2/22.2	10.5/10.5	●/●	–/●	●/●	NatA
	L17 A/B	20.5/20.5	10.9/10.9	–	○/–	○/○	–
	L18 A/B [d]	20.6/20.6	11.7/11.7	–	–	○/○	–
	L19 A/B [d]	21.7/21.7	11.4/11.4	○/–	–	○/○	–
	L20 A/B	20.4/20.4	10.3/10.3	–	–	○/○	–
	L21 A/B	18.2/18.3	10.4/11.2	–	○/–	○/–	–
	L22 A/B	13.7/13.8	5.9/6.0	–	○/–	○/–	–
	L23 A/B [d]	14.5/14.5	10.3/10.3	–	–	●/●	NatA
	L24 A/B	17.6/17.5	11.3/11.4	–	–	○/○	–
	L25	15.7	10.1	○	○	○	–
	L26 A/B	14.2/14.2	11.4/10.5	–	–/○	–/○	–
	L27 A/B	15.5/15.5	10.4/11.2	–	○/–	○/–	–
	L28	16.7	10.5	–	○	○	–
	L29	6.7	12	–	○	–	–
	L30	11.4	9.8	–	○	○	–
	L31 A/B	12.9/13.0	10.0/10.0	–	○/–	○/○	–
	L32	14.8	11.2	–	○	○	–
	L33 A/B	12.1/12.2	11.1/11.1	–	●/–	○/○	–
	L34 A/B	13.6/13.6	11.6/11.6	–	–	–	–
	L35 A/B [d]	13.9/13.9	10.6/10.6	–	–	○/○	–
	L36 A/B	11.1/11.1	12.2/11.6	–	●/ ○	–/○	–
	L37 A/B	9.8/9.7	12.2/12.3	–	–	–	–
	L38	8.8	10.9	–	○	○	–
	L39	6.3	–	–	○	–	–
	L40 A/B [d]	14.5/14.5	10.6/10.6	–	–	–	–
	L41 A/B [d]	3.3/3.3	–	–	–	–	–
	L42 A/B [d]	12.2/12.2	11.4/11.4	–	–/○	–	–
	L43 A/B [d]	10.0/10.0	11.2/11.4	–	–	–	–

40S	S0 A/B	28.0/28.0	4.5/4.5	–	●/–	–	–
	S1 A/B	28.7/28.8	10.0/10.0	●/–	●/–	○/○	NatA
	S2	27.4	10.4	●	●	●	NatA
	S3	26.5	9.4	○	○	○	–
	S4 A/B [d]	29.3/29.3	10.1/10.1	–	○/–	○/○	–
	S5	25	8.6	●	●	●	NatA
	S6 A/B [d]	27.0/27.0	10.4/10.4	–	–	○/○	–
	S7 A/B	21.6/21.6	9.8/9.9	●/–	●/●	●/●	NatA
	S8 A/B [d]	22.5/22.5	10.7/10.7	–	○/–	○/○	–
	S9 A/B	22.4/22.3	10.8/10.1	–	–	–/○	–
	S10 A/B	12.7/12.7	8.7/9.92	–	○/○	○/–	–
	S11 A/B [d]	17.7/17.7	10.8/10.8	–	●/–	●/●	NatA
	S12	15.8	4.5	–	–	–	–
	S13	17	10.4	–	○	○	–
	S14 A/B	14.5/14.6	10.7/11.3	●/–	●/–	●/–	NatA
	S15	16	10.7	–	●	●	NatA
	S16 A/B [d]	15.8/15.8	10.3/10.3	●/–	●/–	●/●	NatA

Table 1 (*continued*)

Subunit	Protein	MW (kDa) [a]	pI [a]	2-DE [b]	TOF-MS [c]	DIGE	NAT
40S	S17 A/B	15.8/15.8	10.5/11.3	–	–	●/–	NatA
	S18 A/B [d]	17.0/17.0	10.3/10.3	–	●/●	●/●	NatA
	S19 A/B	15.9/15.9	9.6/10.5	–	–/○	○/–	–
	S20	13.9	9.5	–	●	●	NatA
	S21 A/B	9.7/9.5	5.8/5.8	–	●/●	●/●	NatB
	S22 A/B	14.6/14.6	9.9/9.9	–	○/–	○/○	–
	S23 A/B [d]	16.0/16.0	11.5/11.5	–	–	–	–
	S24 A/B [d]	15.3/15.3	10.5/10.5	●/–	●/–	●/●	NatA
	S25 A/B	12.0/12.0	10.3/11.1	–	–	○/–	–
	S26 A/B	13.5/13.4	10.8/11.6	–	–	○/–	–
	S27 A/B	8.9/8.9	9.4/9.5	–	○/○	○/–	–
	S28 A/B	7.6/7.6	10.8/11.4	–	●/●	●/–	NatB
	S29 A/B	6.7/6.7	11.1/10.8	–	○/○	–	–
	S30 A/B	7.1/7.1	12.2/12.2	–	○/○	–	–
	S31	17.2	10.7	–	–	–	–
Total	78			18	50	60	

●: N^{α}-Acetylated ribosomal protein. ○: identified ribosomal protein.

[a] The calculated molecular weight and pI of ribosomal proteins were obtained from SWISS-PROT database.

[b] N^{α}-Acetylated ribosomal proteins were identified by 2-DE with an amino acid sequencer (Takakura et al.).

[c] N^{α}-Acetylated ribosomal proteins were identified by MALDI-TOF-MS (Arnold et al.).

[d] These ribosomal proteins A/B are used for duplicated genes that code proteins with identical sequence.

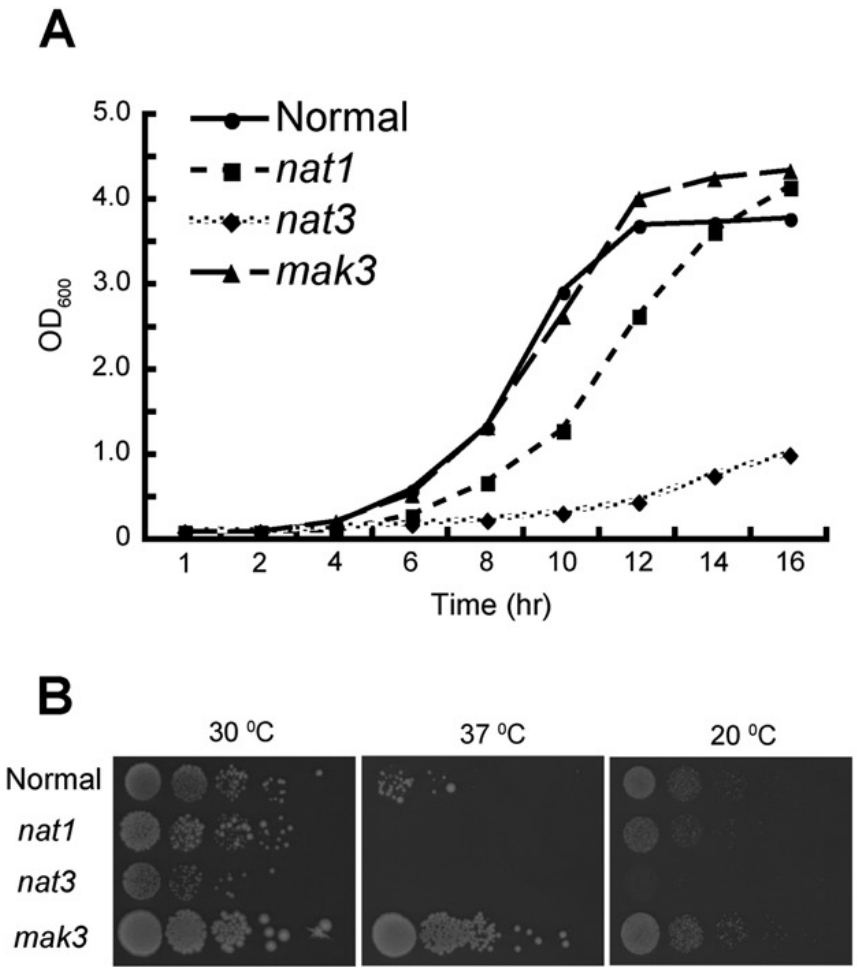
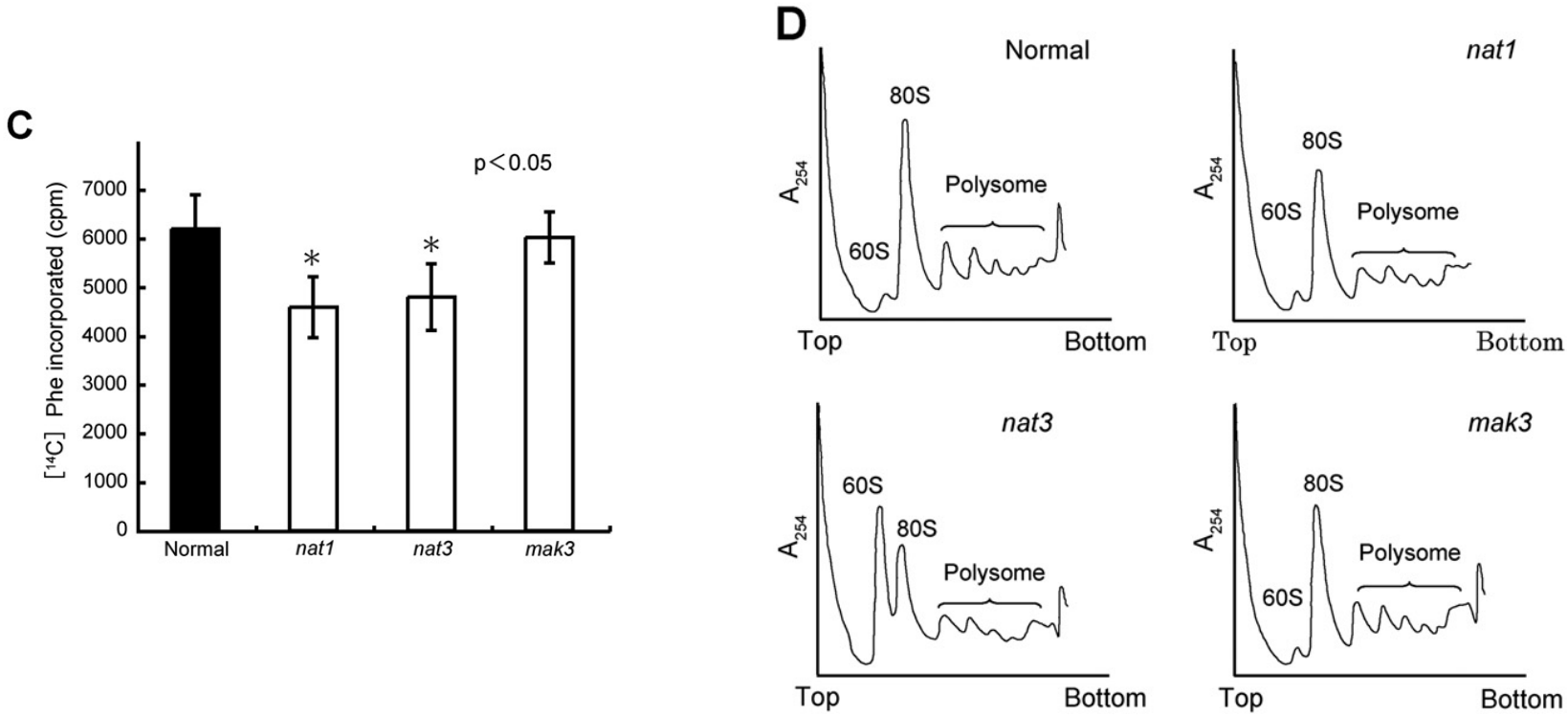


Fig. 2 – The effect of N^{α}-acetylation on cell growth and protein synthesis. (A) Growth curves of the normal strain and the NAT mutants. All strains were cultured in YPD at 30 °C until stationary phase. The absorbance of each culture was measured at 600 nm every 2 h. (B) Effect of three different temperatures (20, 30 and 37 °C) on the growth of the normal strain and the NAT mutants. Freshly grown yeast colonies were suspended in water, and 1/10 dilutions containing the same number of cells were spotted onto YPD plates. Spotted plates were incubated at 20, 30 and 37 °C for 3 to 4 days. (C) Effect of N^{α}-acetylation on polyU-dependent poly



YPD plates. Spotted plates were incubated at 20, 30 and 37 °C for 3 to 4 days. (C) Effect of N^{α}-acetylation on polyU-dependent poly (Phe) synthesis. Purified 80S ribosomes from the normal strain and the NAT mutants were added to assay mixtures containing soluble factor S-100 from the normal strain and radioactive Phe residues, and incubated at 30 °C for 30 min. The radioactivity of the insoluble fraction, a measure of the incorporation of radioactive amino acids, was determined by liquid scintillation counter. The value shown in the figure was calculated by subtracting the value of the activity at 0 min. (D) The polysome profiles of the normal strain and the NAT mutants. Cytoplasmic extracts from the normal and the mutant strains were loaded onto 7–47% sucrose gradients, centrifuged, and fractionated. The fractions were collected from the top to the bottom with continuous A_{254} monitoring.

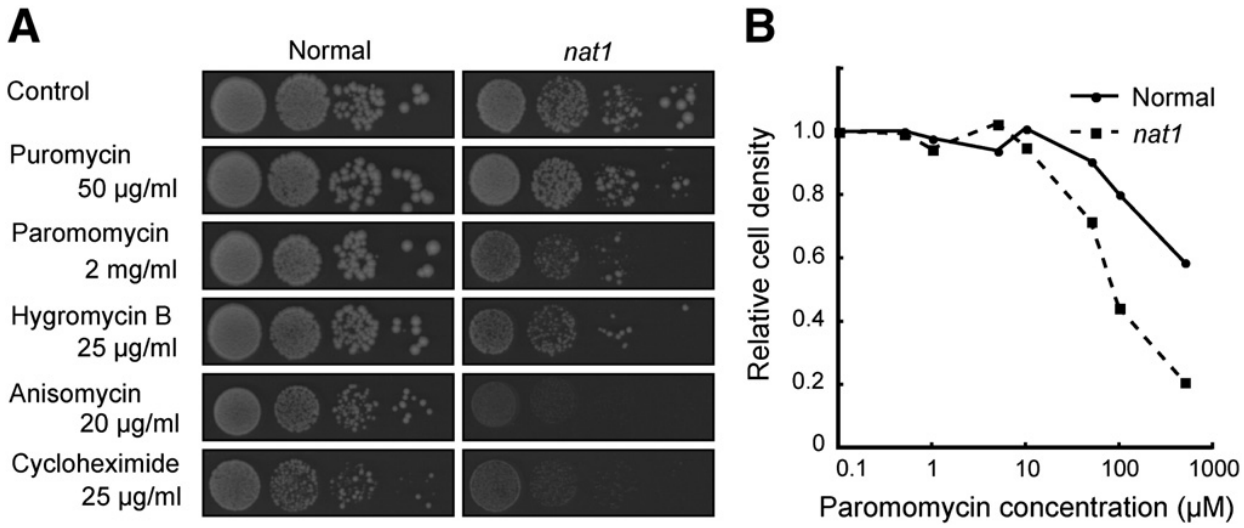


Fig. 3 – The effect of the NatA deletion on sensitivity to translation inhibitors. (A) The effect of various antibiotics on the growth of the normal strain and the *nat1* mutant. Freshly grown yeast colonies were suspended in water, and 1/10 dilutions starting at 0.1 OD_{600} were spotted onto YPD plates containing the indicated antibiotics. Spotted plates were incubated at 30 °C for 4 days. (B) The effect of paromomycin on growth of the normal strain and the *nat1* mutant. Freshly grown yeast colonies were cultured in YPD containing increasing concentrations of antibiotics until the culture without antibiotic reached an OD_{600} of 1–1.5, which was taken as 1.0.

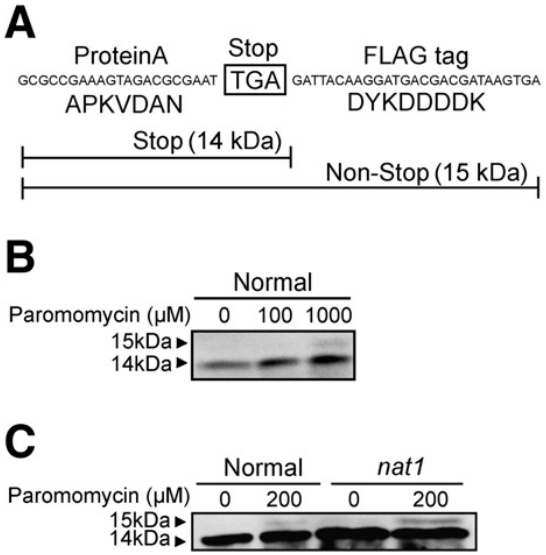


Fig. 4 – The role of ribosomal protein N^{α}-acetylation in translational readthrough activity. (A) The structure of the stop codon readthrough construct used in this study. The 14 kDa protein A fragment is a predominant translation product in the normal strain. The 14 kDa protein A fragment combined with the FLAG tag protein (1 kDa) which resulted in 15 kDa peptide is the mistranslated protein product. (B) Protein production in the normal strain with high concentration of paromomycin induced stop codon readthrough. Cells were grown in YPD containing the indicated concentration of paromomycin. Protein samples were loaded onto 15% SDS-PAGE and detected using Western blot with anti-peroxidase antibody. (C) Comparison of stop codon readthrough activity between the normal strain and the *nat1* mutant.

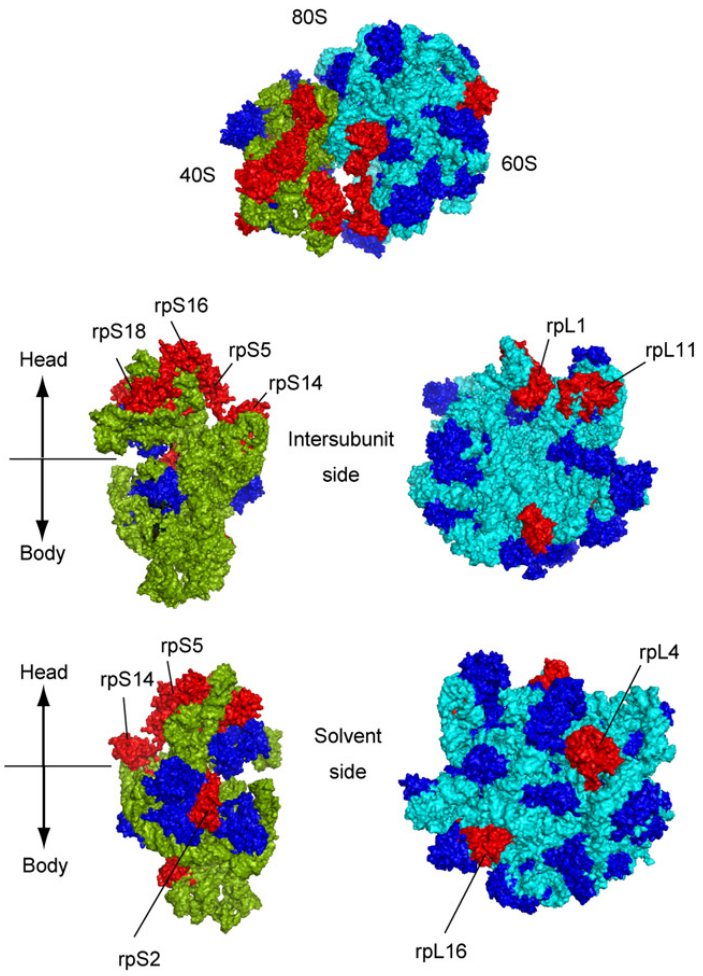


Fig. 5 – Location of NatA N^{α}-acetylated ribosomal proteins on the 80S ribosome structure. The 25S rRNA is in light blue, the 18S rRNA is in green, the non-N^{α}-acetylated ribosomal proteins are in dark blue, and the N^{α}-acetylated ribosomal proteins are in red. The graphic visualization was done with the program PyMol (PDB ID: 1S1H and 1S1I).