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Synthetic Lethal Screen of *NAA20*, a Catalytic Subunit Gene of NatB N-Terminal Acetylase in *Saccharomyces cerevisiae*

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The *Saccharomyces cerevisiae* NatB N-terminal acetylase contains a catalytic subunit Naa20 and an auxiliary subunit Naa25. To elucidate the cellular functions of the NatB, we utilized the Synthetic Genetic Array to screen for genes that are essential for cell growth in the absence of *NAA20*. The genome-wide synthetic lethal screen of *NAA20* identified genes encoding for serine/threonine protein kinase Vps15, 1,3-beta-glucanosyltransferase Gas5, and a catabolic repression regulator Mig3. The present study suggests that the catalytic activity of the NatB N-terminal aceytase is involved in vacuolar protein sorting and cell wall maintenance.

Keywords: N-terminal acetylation, synthetic lethal screen, NatB, Naa20, synthetic genetic array

Introduction

The N-terminal residues of eukaryotic proteins are very frequently modified by N^{α}-terminal acetylation (Nt-acetylation), which occurs in up to ~60% of yeast proteins and 90% of human proteins (Arnesen *et al.*, 2009; Van Damme *et al.*, 2012). In contrast to acetylation of internal lysine residues, which is a reversible modification, Nt-acetylation is seemingly irreversible (Van Damme *et al.*, 2012). This process takes place co-translationally as well as post-translationally (Starheim *et al.*, 2012; Van Damme *et al.*, 2012).

Over the past 60 years, the biological role of Nt-acetylation had largely remained a mystery. However, recent studies have revealed that Nt-acetylation plays a role in a variety of cellular functions including, among others, regulation of protein stability (Jornvall, 1975; Hwang *et al.*, 2010b; Shemorry *et al.*, 2013), protein-protein interactions (Scott *et al.*, 2011), and protein trafficking to organelles (Behnia *et al.*, 2004; Setty *et al.*, 2004; Forte *et al.*, 2011). Initially, the widespread view regarding protein stability was that Nt-acetylation would protect a protein from degradation (Jornvall, 1975; Helbig *et al.*, 2010). However, we previously discovered that Nt-acetylation creates specific protein degradation signals (degrons) that are targeted by a branch of the N-end rule pathway, termed the Ac/N-end rule pathway (Hwang *et al.*, 2010b). In *Saccharomyces cerevisiae*, the Ac/N-end rule pathway involves the Doa10 or Not4 E3 ubiquitin (Ub) ligases, which specifically recognize cellular proteins modified with Nt-acetylated residues and target them for Ub-dependent proteasomal degradation (Hwang *et al.*, 2010b; Shemorry *et al.*, 2013; Kim and Hwang, 2014). The Ac/N-end rule pathway functions in the control of protein quality and stoichiometries of subunits in protein complexes (Shemorry *et al.*, 2013). Another branch of the *S. cerevisiae* N-end rule pathway is the Arg/N-end rule pathway. This involves Ubr1 E3 Ub ligase, which directly recognizes unacetylated N-terminal Leu, Ile, Trp, Tyr, Phe, Arg, His, and Lys residues, thus leading to polyubiquitylation of these proteins and, ultimately, proteasomal degradation (Hwang *et al.*, 2010a; Varshavsky, 2011). Most recently, we demonstrated that the Ac/N-end rule and Arg/N-end rule pathways target Nt-acetylated or unacetylated N-terminal Met residues of cellular proteins if they are followed by a large hydrophobic residue (Leu, Ile, Try, Trp or Phe), respectively (Kim *et al.*, 2014). These findings suggested that the N-terminal Met-hydrophobic (Met-Φ) degradation signal occupies a large new class of N-degrons, which correspond to approximately 15% of total yeast or human proteins (Kim *et al.*, 2014; Kim and Hwang, 2014).

These Nt-acetylation reactions are catalyzed by a group of Nt-acetylase complexes that utilize acetyl-CoA to attach an acetyl group to the α-amino group of a target substrate. In *S. cerevisiae,* the Nt-acetylase complexes are classified according to their substrate specificity and subunit compositions and are comprised of NatA, NatB, NatC, NatD, and NatE (Polevoda and Sherman, 2003; Starheim *et al.*, 2012; Van Damme *et al.*, 2012; Kalvik and Arnesen, 2013). The NatA complex consists of a catalytic subunit Naa10 (Ard1) and an auxiliary subunit Naa15 (Nat1) to facilitate the Nt-acetylation of proteins with an N-terminal Ser, Ala, Thr, Gly, Val, or Cys residue. The NatB complex contains a catalytic subunit Naa20 (Nat3) and an auxiliary subunit Naa25 (Mdm20) to N-terminally acetylate proteins with Met-Asn, Met-Asp, Met-Gln, or Met-Glu at their N-termini. The NatC complex carries a catalytic subunit Naa30 (Mak3) and auxiliary subunits Naa35 (Mak10) and Naa38 (Mak31), and is responsible for the Nt-acetylation of cellular proteins with an N-terminal Met that is followed by a hydrophobic residue at position 2. NatD contains only a catalytic subunit, Naa40 (Nat4), and functions specifically in the Nt-acetylation of histones H2A and H2B. NatE is a complex of Naa50 (Nat5) and NatA, which facilitates the Nt-acetylation of several proteins with

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Table 1. ***S. cerevisiae*** **strains used in this study**

Strains	Genotypes	Reference/source
BY4741	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
Y7092	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+*	Tong and Boone (2006)
CHY719	*naa20Δ::natNT2* in Y7092	This study
CHY2073	*vps15Δ::kanMX4* in BY4741	Yeast deletion mutant collection
CHY2074	*naa20Δ::kanMX4* in BY4741	Yeast deletion mutant collection
CHY2075	*gas5Δ::kanMX4* in BY4741	Yeast deletion mutant collection
CHY2076	*gga1Δ::kanMX4* in BY4741	Yeast deletion mutant collection
CHY2077	*mig3Δ::kanMX4* in BY4741	Yeast deletion mutant collection
CHY 2092	CHY2074 with *NAA20* in pRS316	This study
CHY 2094	*vps15Δ::hphNT1* in CHY2074 with *NAA20* in pRS316	This study

a hydrophobic residue at position 2 (Starheim *et al*., 2012; Van Damme *et al*., 2012; Kalvik and Arnesen, 2013).

Of these Nt-acetylase complexes, the present study initially aimed to uncover new cellular functions of the NatB complex in order to understand its role in the N-end rule pathway. *S. cerevisiae* cells lacking NatB activity (*naa20Δ* or *naa25Δ*) were shown to have severe phenotypes with sensitivities to temperature, osmotic stress, DNA damage, and chemical drugs (Polevoda *et al*., 2003; Van Damme *et al*., 2012). In addition, these cells were defective in mating, morphology, mitochondrial division, vacuolar segregation, cell polarity, and so on (Polevoda *et al*., 2003; Singer and Shaw, 2003). Furthermore, protein phosphorylation in naa20Δ cells were elevated despite minimal perturbation in protein expression levels. Therefore, the pleiotropic phenotypes of *S. cerevisiae* due to the loss of NatB activity in *naa20Δ* or *naa25Δ* cells indicates that NatB-dependent Nt-acetylation is likely to be involved in diverse cellular processes (Polevoda *et al*., 2003; Singer and Shaw, 2003; Van Damme *et al*., 2012). Two-dimensional gel electrophoresis and global proteomic approaches have identified more than 110 *S. cerevisiae* NatB substrates (Van Damme *et al*., 2012), including actin (Act1), tropomyosin 1 (Tpm1), ribosomal proteins S21 and S28 (Arnold *et al*., 1999), a ribonucleotide reductase subunit Rnr4 (Polevoda *et al*., 1999), and proteasome subunits Pre3, Rpt3, and Rpn11 (Kimura *et al*., 2000, 2003), a carboxypeptidase inhibitor Tfs1 (Caesar and Blomberg, 2004). However, the mechanism by which NatB controls this variety of cellular processes remains unclear.

Here, we carried out a genome-wide synthetic genetic array (SGA) analysis and identified synthetic lethal (SL) interactions of *NAA20* with the serine/threonine protein kinase *VPS15* (Stack *et al*., 1995), 1,3-beta-glucanosyltransferase *GAS5* (Ragni *et al*., 2007), and a catabolic repression transcription regulator *MIG3*.

Materials and Methods

Yeast strains, media, and genetic techniques

S. cerevisiae strains and oligomers used for this study are listed in Tables 1 and 2, respectively. Yeast strain construction and transformation were carried out based on standard techniques (Sherman, 1991). The yeast culture media included YPD (1% yeast extract, 2% peptone, 2% glucose), SD (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose), and synthetic complete (SC) (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose, and a drop-out mixture of compounds required for cell growth), SD-MSG (0.17% yeast nitrogen base, 0.1% L-glutamic acid, 0.2% amino acid supplement mixture required for cell growth), and enriched sporulation (1% potassium acetate, 0.1% yeast extract, 0.05% glucose, 0.01% amino acid supplement mixture required for sporulation) media (Tong and Boone, 2006). The *NAA20* gene

Table 2. **Oligomers used in this study**

Primers	Sequences
OCH1229	5′-ATGACAACAATTCAGCCTTTTGAACCTGTCGATTTATTCAAAACTAACAATTCGTACGCTGCAGGTCGAC-3′
OCH1230	5′-TTAAAATCTTACATCATGTGGATAGCATTTATGGCTTCTTCCATCCGGCCGATCGATGAATTCGAGCTCG-3′
OCH1231	5′-TTCAAGGAAAGAGACAGGAGGATT-3′
OCH1232	5′-TTATGTTCTGAGTATGAGGACGAG-3′
OCH2168	5′-GATGTCCACGAGGTCTCT-3′
OCH2169	5′-CATATAAATCAGCATCCATGT-3′
OCH2170	5′-CGTCACTCATGGTGATTTCTC-3′
OCH2171	5′-CGGTGTCGGTCTCGTAG-3′
OCH2186	5′-ATGGGGGCACAATTATCACTAGTGGTCCAAGCATCACCTTCCATAGCCATTCGTACGCTGCAGGTCGAC-3′
OCH2187	5′-GTAACCAAATTTCTGAAATAATCATAAATGAAAGTGTAGAAATAATCGGGGATCGATGAATTCGAGCTCG-3′
OCH2188	5′-GCAGACAAGTTGAATTGGAGA-3′
OCH2189	5′-AAGGTACTATTGGTGCAAGTG-3′
OCH2190	5′-ACTGGATCCTCTGTGGACACGCAACTTTCTAG-3′
OCH2191	5′-AACCTCGAGAACAGAGGAAACAGAGGAAGAGG-3′

Table 3. Genes showing synthetic lethal interaction with *NAA20*

ORF	Gene	Functions of gene product
YBR097W	*VPS15*	Serine/threonine protein kinase involved in vacuolar protein sorting
YOL030W	*GAS5*	1,3-beta-glucanosyltransferase involved in the cell wall maintenance
YER028C	*MIG3*	Transcriptional regulator for glucose repression

in *S. cerevisiae* strain Y7092 (Tong and Boone, 2006) was disrupted in order to construct the query strain CHY719 (*naa20Δ::natNT2*) by PCR-mediated gene targeting using a primer pair (OCH1229/OCH1230) and the natNT2 module (Janke *et al.*, 2004). The deletion of *NAA20* was confirmed by genomic PCR using a primer pair (OCH1231/OCH1232).

SGA analysis

We employed the SGA methodology as described previously (Tong and Boone, 2006), with slight modifications. All replica pinning steps were performed using a SGA-RoTor robot system (Singer Instruments). In brief, 5 ml of overnight cultured CHY719 (*naa20Δ::natNT2*) cells were poured onto new YPD plates and spread to form a lawn. The plates were dried and incubated at 30°C for 1 day. The deletion strains with a BY4741 genetic background were pinned onto fresh YPD plates with 384 colonies per plate. Then, the deletion mutants were overlaid on top of query strain CHY719. The plates were incubated overnight at 30°C for mating and diploid cells were selected by replica-plating on YPD medium containing G418 (200 mg/ml) (AG Scientifics, USA) and clonNAT (100 mg/ml) (Werner BioAgents, Germany). Thereafter, arrays were pinned onto enriched sporulation medium. After a 10-day incubation at 22°C, spores were germinated by pinning onto haploid selection medium (SD -histidine + thialysine + canavanine) and incubated at 30°C for 2 days to select for the growth of *MAT**a*** spore progeny. Meiotic progeny with the G418 resistance locus were selected by replica-plating onto SD-MSG medium supplemented with thialysine, canavanine, and G418, and incubated overnight at 30°C. Finally, *MAT**a*** meiotic progenies were selected on medium containing G418 and clonNAT for 2 days at 30°C. Synthetic lethal colonies were identified directly by eye.

Random spore analysis

To confirm putative interactions identified in the above SGA technique, *MAT**a*** meiotic progeny spores from heterozygous diploid (*vps15Δ*/+ *naa20Δ*/+, *gas5Δ*/+ *naa20Δ*/+, *mig3Δ*/+ *naa20*/+) strains were diluted in sterile distilled water and incubated at 30°C for 3 days on solid haploid selection media consisting of SD/MSG – His/Arg/Lys + canavanine/thialysine ± G418, clonNAT, or G418/clonNAT. Double mutant progenies were selected on medium containing G418 and clonNAT and the cells showing no growth were scored as synthetic lethal with *NAA20*.

Strain confirmation of the yeast deletion collection

Genomic DNAs from the 3 deletion mutant strains described above were amplified by PCR using primer pair specific to gene deletion set and the KanMX4 module (OCH2168/OCH2169, OCH2170/OCH2171). The barcodes of the amplified PCR products were sequenced and used as a query to confirm the specific gene deletion of the mutants.

Construction of *naa20Δ vps15Δ* yeast strains carrying an episomal *NAA20* plasmid

To construct pCH2133, the *NAA20* gene was PCR-amplified from yeast genomic DNA (using primer pair OCH2190/OCH2191), digested with *Xho*I/*Bam*HI, and cloned into *Xho*I/*Bam*HI-cut pRS316 vector. The amplified PCR product contained the *NAA20* coding region flanked by 500 bp of upstream and 300 bp of downstream sequences. The resulting pCH2133 plasmid was confirmed by DNA sequencing. To construct the *naa20Δ vps15Δ* strain that expressed *NAA20* from a CEN-based low copy pRS316, pCH2133 was first introduced into the CHY2074 (*naa20Δ*) strain, yielding CHY2092 (*naa20Δ* cells expressing *NAA20* in pRS316). Thereafter, the *VPS15* gene was disrupted in CHY2092 through PCR-mediated gene targeting using primer pair OCH2186/OCH2187 and the hphNT1 module (Janke *et al.*, 2004). The correct gene deletion was confirmed by PCR using primer pair OCH2188/OCH2189.

Cell growth assays

To examine the effects of stressors on cell growth, *S. cerevisiae* strains BY4741 (WT), CHY2073 (*vps15Δ*), CHY2074

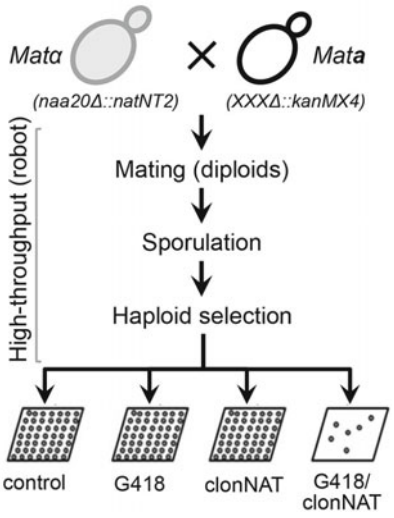

Fig. 1. Schematic representation of the SGA technique. A clonNAT-resistant query *MAT*α (*naa20Δ::natNT2*) strain was arrayed using a high throughput robot (SGA RoTor) and crossed to G418-resistant *MAT**a*** (*xxxΔ::kanMX4*) mutants. After mating, the heterozygous diploids were sporulated, followed by consecutive haploid selections. The resulting *MAT**a*** meiotic progenies were grown on G418, clonNAT, or G418/clonNAT media for the selection of *xxxΔ*, *naa20Δ*, or *xxxΔ naa20Δ* cells, respectively. Also refer to 'Materials and Methods' for further details.

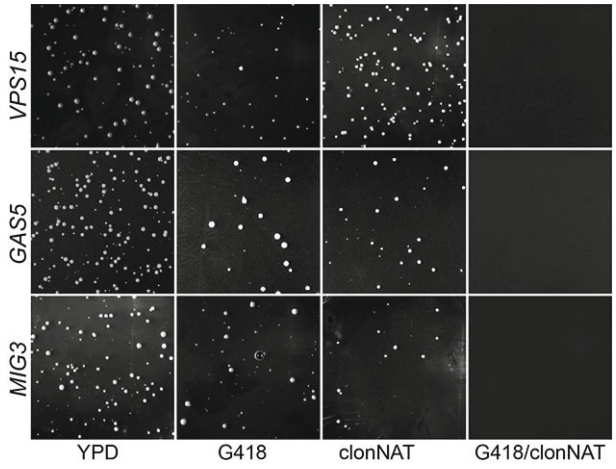

Fig. 2. Random spore analysis for the confirmation of synthetic lethality. Heterozygous diploid (*vps15Δ::kanMX4/+ naa20Δ::natNT2/+*), (*gas5Δ::kanMX4/+ naa20Δ::natNT2/+*), (*mig3Δ::kanMX4/+ naa20Δ::natNT2/+*) strains were sporulated. The indicated meiotic progenies were plated onto haploid selection media (control) containing either clonNAT and/or G418. The plates were incubated at 30°C for 3 days.

(*naa20Δ*), CHY2075 (*gas5Δ*), CHY2076 (*gga1Δ*), or CHY2077 (*mig3Δ*) were grown in YPD medium to A_{600} of ~1.0. Equal volumes of the resulting cultures were serially diluted 5-fold and thereafter spotted onto YPD plates containing various salts (1 M KCl, 1 M NaCl, 0.3 M $CaCl_2$), or 5 mg/ml of Calcofluoro White (CW, used for cell-wall perturbation), followed by incubation at 30°C for 2 days. To examine the strains' response to heat stress, the above-described serially diluted cells were spotted onto YPD plates and incubated at 39°C for 2 days. For determining ultraviolet (UV) sensitivity, the dilutions were irradiated with UV light (for the induction of DNA damage) on a clean bench for 1 min and then incubated at 30°C for 2 days. For the analysis of carbon source utilization, the cells were plated onto YPD, YPE (2% ethanol in YPD instead of glucose, or YPGal (2% galactose in YPD instead of glucose) plates. In order to verify the synthetic lethal interaction between *NAA20* and *VPS15*, ~10^4 cells of strain CHY2092 (*naa20Δ* cell expressing *NAA20* in pRS316) or CHY2094 (*naa20Δ vps15Δ* cells expressing *NAA20* in pRS316) were grown on either SC-Ura or FOA+SC plates containing 5-fluoroorotic acid (1 mg/ml) (Zymo Research) and uracil (20 mg/ml) for 5 days.

Results

Genome-wide synthetic lethal screen of Naa20

Naa20 is a catalytic subunit of NatB Nt-acetylase complex. To gain new insights into unknown physiological functions of the NatB Nt-acetylase, we employed the SGA analysis, which enables the systematic creation of double mutants and allows for a large-scale characterization of synthetic lethal interactions. Using this technique, we screened nonessential *S. cerevisiae* genes to determine which mutations caused lethality when in combination with the *NAA20* deletion. First, we constructed a query *naa20Δ MAT*α strain that carried a clonNAT-selection marker. The query *Mat*a strain was systematically mated with the arrayed *MAT**a*** deletion mutants in 4847 genes harboring G418 as a selection marker. The resulting G418- and clonNAT-resistant diploid strains were isolated, sporulated, and selected on specific haploid-specific media (see details in 'Materials and Methods'). Thereafter, the haploid strains showing no growth on selective media containing G418 and clonNAT were scored as synthetic lethal growth phenotypes (Figs. 1 and 2). The genome-wide SGA analysis identified 34 presumptive mutant candidates showing genetic interactions when combined with the deletion of *NAA20*. Using random spore analysis, we found that, indeed, 3 of the 34 genes showed synthetic lethal interactions for *NAA20* (Fig. 2). The identified genes included the serine/threonine protein kinase *VPS15* (Stack *et al.*, 1995), 1,3-beta-glucanosyltransferase *GAS5* (Ragni *et al.*, 2007), and a transcriptional regulator, *MIG3* (Westholm *et al.*, 2008) (see also Table 3).

Phenotypes of synthetic lethal interaction genes with *NAA20*

naa20Δ cells were previously reported as having impaired growth phenotypes under broad range of stressors such as heat, high salt concentrations, DNA-damaging agents, and non-fermentative carbon sources (Polevoda *et al.*, 2003; Van Damme *et al.*, 2012). To examine whether *vps15Δ*, *gas5Δ*, or *mig3Δ* cells shared common phenotypes with *naa20Δ* cells, exponentially growing *S. cerevisiae* cells were serially diluted 5-fold, spotted onto YPD media containing the appropriate selective agents, and incubated at either 30°C or 39°C (Figs. 3–5). Since random spore analysis of a *gga1Δ* heterozygous mutant isolated from the first positive hits of the SGA screen showed that *GGA1* had no synthetic lethal interaction with *NAA20*, this mutant was used as a negative control in the growth assay. Among the mutant cells tested, *vps15Δ* and *naa20Δ* cells were hypersensitive toward high salt concentrations (1 M KCl, 1 M NaCl, 0.3 M $CaCl_2$) and elevated tem-

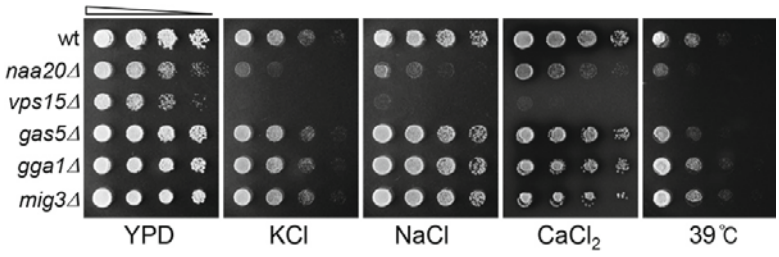

Fig. 3. Effects of salt or temperature on cell growth. Equal amounts of cells from wild-type, *vps15Δ*, *gas5Δ*, and *gga1Δ* strains were 5-fold serially diluted and spotted on YPD plates containing 1 M KCl, 1 M NaCl, or 0.3 M $CaCl_2$ and incubated at 30°C for 2 days. For the temperature sensitivity assay, the YPD plate was incubated at 39°C for 3 days. Since random spore analysis showed that *GGA1* had no synthetic lethal interaction with *NAA20*, the *gga1Δ* mutant was used as a negative control for the growth assay.

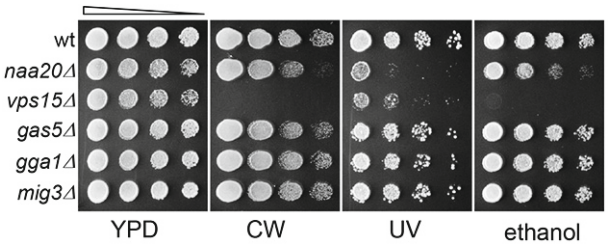

Fig. 4. Effects of various stressors on cell growth. Equal amounts of cells from wild-type (BY4742), *vps15Δ*, *gas5Δ*, and *gga1Δ* strains were 5-fold serially diluted and spotted on YPD plates containing 5 mg/ml of Calcofluoro White (CW) or 5% ethanol (EtOH) and incubated at 30°C for 2 days. The UV-sensitivity assay was described in the 'Materials and Methods'. The *gga1Δ* mutant was used as a negative control for the growth assay.

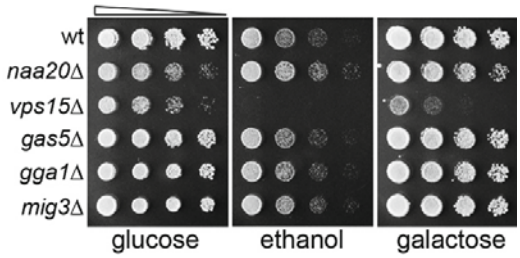

Fig. 5. Effects of carbon sources on cell growth. Equal amounts of cells from wild-type (BY4741), *vps15Δ*, *gas5Δ*, and *gga1Δ* strains were 5-fold serially diluted and spotted on YPD, YPE, or YPG plates containing 2% glucose, ethanol, or galactose, respectively. The *gga1Δ mutant* was used as a negative control for the growth assay.

perature (39°C), whereas wild-type, *gga1Δ*, *gas5Δ*, and *mig3Δ* grew comparably to one another (Fig. 3). In addition, *naa20Δ* and *vps15Δ* cells were highly hypersensitive to Calcofluoro white (for the cell-wall perturbation), UV-irradiation (for the induction of DNA damages), and ethanol (for the perturbation of cell membrane and induction of oxidative stresses), while *gas5Δ* and *mig3Δ* cells grew comparably with the wild-type cells (Fig. 4). Next, we compared the strains' growth phenotypes on yeast extract-peptone media containing glucose (YPD), ethanol (YPE), or galactose (YPGal) as carbon sources. *naa20Δ* cells grew as well as wild-type on YPGal and YPE media, whereas *vps15Δ* cell growth was remarkably diminished (Fig. 5). Although *naa20Δ* cells were reported to be defective in growth on YPE or YPGal media, it was also noted that their growth phenotypes varied depending upon the genetic background (Polevoda *et al.*, 2003). In accordance with the results of the current study (Fig. 5), wild-type and *naa20Δ* cells did not show noticeable growth differences on YPGal medium at 30°C (Van Damme *et al.*, 2012). Therefore, the carbon source-dependent slow growth phenotypes of *naa20*Δ seemed to be significantly affected by the varying genetic backgrounds.

Synthetic lethality of *naa20Δ vps15Δ* cells on a FOA-counter-selection

Since an inherent aspect of the SGA procedure might kill double mutants (van Welsem *et al.*, 2008), we wanted to further verify the synthetic lethally of the *naa20Δ vps15Δ* double mutant. To this end, we disrupted *VPS15* through PCR-mediated gene targeting in CHY2092 (*naa20Δ*) cells that expressed the *NAA20* from a *URA3*-marked low copy plasmid. The resulting CHY2094 (*naa20Δ vps15Δ*) cells contained an episomal *NAA20* plasmid. Both CHY2092 and CHY2094 cells were incubated on SC-Ura plates to determine the rates of cell growth under normal conditions or on plates containing 5-fluoroorotic acid and uracil (SC+FOA) for expelling the *URA3*-containing plasmid as a counter-selection. The CHY2094 (*naa20Δ vps15Δ*) cells carrying the episomal *NAA20* plasmid grew slightly slower on SC-Ura3 plates compared to CHY2092 (*naa20Δ*) cells. Importantly, despite the comparable growth of CHY2092 (*naa20Δ*) cells, the CHY2094 (*naa20Δ vps15Δ*) cells did not form colonies on the FOA+SC plate, providing further evidence of the synthetic lethality between *NAA20* and *VPS15*.

Discussion

In the present study, we utilized SGA methodology to demonstrate that *NAA20* genetically interacts with *VPS15*, *GAS5*, and *MIG3*. We also observed that *naa20*Δ and *vps15Δ* cells shared nearly similar growth phenotypes in response to numerous stressors. Nt-acetylation of actin and tropomyosin by NatB has been shown to contribute to the stability of actin cables (Polevoda *et al.*, 2003; Singer and Shaw, 2003). Vps15 is a Ser/Thr protein kinase that is associated with a phosphatidylinositol 3-phosphate kinase, Vps34 (Stack *et al.*,

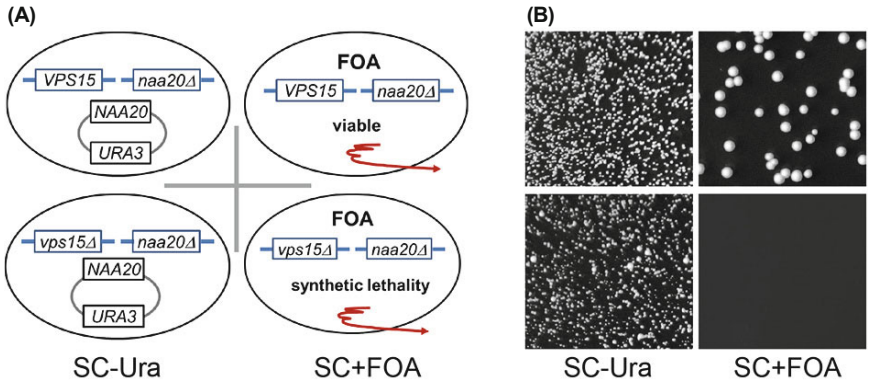

Fig. 6. A FOA-counter-selection of *naa20Δ* cells or *naa20Δ vps15Δ* mutant cells carrying an episomal *NAA20* plasmid. (A) Schematic representation of the FOA-selection strategy. (B) ~10^4 of cells from *naa20Δ* and *naa20Δ vps15Δ* strains carrying the *NAA20* on a *URA3*-based low copy plasmid were grown for 5 days on SC-Ura or SC+FOA plates, respectively.

1995). Interestingly, the functions of Vps15 include vacuolar protein sorting and trafficking, and autophagy (Stack *et al.*, 1995; Lindmo *et al.*, 2008). Several lines of evidence supported that actin cables are required for vacuolar protein sorting, autophagy processes (Bonangelino *et al.*, 2002; Monastyrska *et al.*, 2009), and a wide-range of cellular responses to heat or salt stresses and DNA damage (Holubarova *et al.*, 2000; Mounier and Arrigo, 2002). Taken together, the present results indicate that both Naa20 and Vps15 may regulate the processes of actin and/or the cytoskeleton.

Although *NAA20* genetically interacted with *GAS5* and *MIG3*, *gas5Δ* and *mig3Δ* cells exhibited very few growth defects in comparison to wild-type cells under various stress conditions. These observations may be explained by the fact that Gas5 and Mig3 have redundant proteins in *S. cerevisiae*. Gas5 is one of five *S. cerevisiae* Gas proteins that are thought to be 1,3-beta-glucanosyltransferases responsible for cell wall maintenance (Ragni *et al.*, 2007). Mig3 and its paralogs, Mig1 and Mig2, play redundant roles in the catabolic repression of multiple genes (Westholm *et al.*, 2008; Lewis and Gasch, 2012). Therefore, the present findings suggest that NatB may regulate the cellular functions of the redundant proteins of Gas5 or Mig3 to additively express the full activities in cell wall maintenance or catabolic repression, respectively.

The previous SGA screens of $GlC7^{E101Q}$ (a Glc7 catalytic mutant), *MAD2* (a spindle checkpoint component), *NUP133* (a nuclear porin), *RAD27* (a structure-specific endonuclease), and *RTC3* (a protein involved in RNA metabolism) identified *NAA20* as their synthetic genetic interacting gene (Savchenko *et al.*, 2005; Daniel *et al.*, 2006; Logan *et al.*, 2008; van Welsem *et al.*, 2008). However, those genes were not isolated from the current *NAA20* SGA screen. These discrepancies might result from the heterogeneous genetic backgrounds or secondary mutations of bait or deletion-collection strains and/or inherent experimental noise during the screening procedures. Therefore, more careful examinations (such as a stringent tetrad analysis) of the SGA screen data obtained thus far will be necessary to exclude the possible false positive interactions.

The Ac/N-end rule pathway controls protein quality and subunit stoichiometries (Shemorry *et al.*, 2013). Recent quantitative proteomic analysis identified ~110 NatB substrates (Van Damme *et al.*, 2012). However, it is predicted that the NatB complex is likely to be capable of Nt-acetylating ~15% of all *S. cerevisiae* proteins beginning with Met-Gln, Met-Asn, Met-Glu, or Met-Asp at their N-termini (Van Damme *et al.*, 2012) for production of the bulk of Ac/N-degrons (Hwang *et al.*, 2010a). As a result, the severely deleterious phenotypes of *naa20Δ* cells may result from perturbed degradation of a large fraction of Nt-acetylated proteins. Additional studies are currently underway to further understand the relevance of the Ac/N-end rule pathway to the genetic interaction of *NAA20* with *VPS15*, *GAS5*, and *MIG3*.

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