SUBCELLULAR LOCALIZATION OF ARABIDOPSIS Nᵅ-ACETYLTRANSFERASE 60 BY TRANSIENT EXPRESSION IN TOBACCO LEAF

DINH VAN TRINH
Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City
dinhvantrinh@iuh.edu.vn

Abstract. Protein Nᵅ-terminal acetylation (NTA) catalyzed by Nᵅ-acetyltransferases (NAT) is one of the most common protein modifications in eukaryotes, occurring on approximately 50-70% of yeast and 80-90% of human soluble proteins. NAT has been shown to play a critical role in the functioning of protein such as stability, interaction and targeting. Recently, Nᵅ-acetyltransferase 60 (Naa60 or NatF) has been identified in higher eukaryotes. In human Naa60 is reported to be anchored to the Golgi and responsible for NTA of both cytosolic and membrane proteins. In plant, however, the localization of Naa60 has not been thoroughly addressed. Therefore, this study foccuses on the subcellular localization of Arabidopsis Naa60. Computational analysis of the Naa60 amino acid sequence using PredictProtein reveals potential membrane localization with two amphipathic α-helices at the C-terminus. Transient expression of Arabidopsis Naa60 fused with EYFP at the N- and C- ends in tobacco leaf confirms its localization to the plasma membrane. Co-expression with the RFP plasma membrane marker suggests that Naa60 also localize to the tonoplast.
Keywords. Nᵅ-acetyltransferase, *Arabidopsis thaliana*, Naa60, subcellular localization, leaf-infiltration

1. INTRODUCTION

N-terminal acetylation of protein (NTA) is one of the most common modifications occurred in higher eukaryotes. Remarkably, 50-70% of yeast, 80-90% of human and plant cytosolic proteins are found to be N-terminally acetylated [1, 2]. Single cell prokaryotes such as bacteria and archea appear to have lower level of N-terminally acetylated proteins with 5% and 10%, respectively [3, 4]. The addition of acetyl group to the N-termini affects protein interactions, subcellular localization, protein folding and degradation [5-9]. Recent studies also show the crucial role of NTA in many human pathologies such as cancer, neurodegenerative disorders and genetic diseases like Lenz microphthalmia syndrome [10-12].

NTA is catalized by a group of enzymes called Nᵅ-acetyltransferases (NAT) which belong to the GNAT family protein (GCN5-related N-acetyltransferase) [13]. So far, seven types of NAT have been identified in plants and alphabetically named NatA-NatG according to their substrate specificity and subcellular localization [14]. Each NAT complex normally consists of two subunits, the catalytic subunit (Naa10-NatA, Naa20-NatB, Naa30-NatC, Naa40-NatD, Naa50-NatE, Naa60-NatF, Naa70-NatG) transfers the acetyl group of Acetyl-coA to the α-amino acid of protein and the auxiliary subunit (Naa15-NatA, Naa25-NatB, Naa35-NatC) anchors catalytic subunit to the ribosome. The auxiliary subunit is essential for the function of NatA, NatB and NatC, depletion mutants of Naa15, Naa25 or Naa35 results in lethal or deficient growth of plants due to lack of NAT activity [15-17]. However, in the case of other NATs, the auxiliary subunit does not exit nor seems nesscessary for its activity [18].

N-terminal acetylation is mainly known as co-translational modification of protein and takes place in the cytosol. In this scenario, the acetylated amino acid is the initial methionine or the adjacent residue after cleaving off methionine by methionine aminopeptidase [19]. Recent findings have reveled numerous proteins N-terminally acetylated in organelles confirming the post-translational acetylation of proteins [2]. Subsequently, Naa60/NatF, the first Golgi-localized NAT has been characterized in human [20]. Another newly identifed NAT, Naa70/NatG is revealed to be localized to the plant chloroplast [21].

Subcellular localization plays an important role in the way of NATs modifying their target substrates. Four NAT complexes, NatA-NatC and natE, localize to the cytosol and N-terminally acetylated cytosolic proteins at the time amino acid sequences are translated. NatA co-translationally acetylates N-termini that start with small amino acid such as Alanine, Serine, Threonine, Cystein, Valine and Glycine [22]. NatB acetylates Methionines follow by acidic/hydrophilic residues (MQ-, ME-, MN-, and MD-) while NatC substrates are Methionines with adjacent hydrophobic/amphipathic residues (MF- MI-, ML- MV-, MW-,

MH- and MM-) [22]. On the other hand, NatD exclusively subcellular localizes to the nucleus and known to acetylate protein histones H2A and H4 [23]. The other organellar NAT, NatG, is responsible for post-translationally acetylation of chloroplast proteins after cleaving off the transit peptide [20, 21].
The Naa60/NatF is the most recently identified N^{α}-acetyltransferase in human and plant. The human Naa60 possesses two α-helices the the C-teminus which binds directly to the lipid bilayer of the membrane via hydrophobic and electrostatic interactions [24]. These two amphipathic α-helice indicates that Naa60 possibly localizes to the plasma membrane or any inner membrane system in the cell. In the case of human, Naa60 is anchored to the Golgi and N-teminally acetylates transmembrane proteins [24]. On the other hand, plant Naa60 is attached to the plasma membrane and plays a vital role in the high salt stress response [25]. However, the amino acid sequence of Naa60 is not analized and other localization possibility has not been addressed.
In this study, we investigate the subcellular localization of *Arabidopsis* Naa60 (AtNaa60) using computational analysis and experimental expression of Naa60 fused with EYFP (Enhanced Yellow Fluorescent Protein) in tobacco leaf. Our data show that AtNaa60 is membrane bound protein with dual localization to the plasma membrane and tonoplast.

2. MATERIALS AND METHODS

2.1 Materials

Arabidopsis thaliana ecotype Col-0 (family: Brassicaceae, order: Capparales, class: Dicotyledonae, subdivision: Angiospermae) and *Nicotiana benthamiana* (family: Solanaceae, order: Solanales, class: Dictyledoneae, subdivision: Angiospermae) were obtained from the Department of Plant Molecular Biology, University of Heidelberg, Germany.
Bacterial strains *E. coli* XL1-blue, DH5α and *Agrobacterium tumefaciens* GV3101 were obtained from the Department of Plant Molecular Biology, University of Heidelberg, Germany.

2.2 Total mRNA extraction and cDNA synthesis

Approximately 100mg of leaves was used for total RNA extraction using RNeasy Plant Mini Kit (Quiagen) according to the manufacturer's protocols. RNA concentration was determined using NanoDrop 2000 spectrophotometer.
cDNA was synthesized from 2µg of total RNA extract using RevertAid™ H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) according to the manufacturer's protocols.

2.3 Polymerase Chain Reaction (PCR) and plasmid construction

Specific DNA fragment of *Arabidopsis* Naa60 was amplified by Phusion DNA polymerase (Finnzymes, Espoo, Finland) using MasterCycler, cDNA was used as a template. 18 nucleotides at the 5' end and 23 nucleotides at the 3' end of AtNaa60 were used to design specific primers. attB1 and attB2 sequences were added into primers to be suitable for GATEWAY cloning system. Oligo Calc was used to check for suitable Tm and eliminate any hairpin possibility. Sequences of a forward primer and two reverse primers for C-terminal and N-terminal fusion were describes bellow.
GW-Naa60-for: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTgcATGTCGCGTTTTCCCCGT-3', GW-Naa60-revN: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTcCTATACGTAATCATACCCG GATG-3', GW-Naa60-revC: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTcTACGTAATCAT ACCCGGATGAT-3'.
The PCR components and programs were set up according to manufacturer's protocol as shown bellow (initial denaturing: 98°C for 1 min, denaturing: 98°C for 15 seconds, annealing: 58°C for 20 seconds, elongation: 72°C for 1 min, final elongation: 72°C for 7 min, repeated for 35 cycles).
The amplified DNA products were purified using PCR Clean Up Kit® (Macherey-Nagel, Düren) before cloned into Entry vector and subsequently into destination vectors (pB7YWG2 and pB7WGY2) following the manufacturer's protocols. Before cloning into destination vectors, inserted fracments in the Entry vector were sequenced (Sigma Sequencing Service) to confirm the correct amplification during PCR. The final construcs (pB7YGW2-NAA60 and pB7WGY2-NAA60) were then transformed into *Agrobacterium tumefaciens* strain GV3101 using heat-shock transformation.

2.6 Tobacco leaf infiltration

One colony of *Agrobacterium tumefaciens* (strain GV3101) carrying binary vector was grown in 5 ml of LB medium supplemented with appropriate antibiotics (25 µg/ml Rif, 30 µg/ml Gent and 50 µg/ml Strep)

overnight (16-18h) at 28°C, shaking 200 rpm. The overnight culture was diluted 1/10 with fresh LB medium containing antibiotics and grown at 28°C, shaking 200 rpm to get OD600nm 0.8-1 (about 3-4h). Bacteria were pelleted by centrifuging for 10 min, 5000 rpm at 4°C then washed twice with ice cold sterile water. Finally, bacteria were re-suspended with ice cold water to get an OD600nm 0.8. *Nicotiana benthamiana* plants were kept 100% humidity for at least 1 hour before infiltration. The bacteria were infiltrated into the leaves using 1ml syringe without a needle. The expression of transgene was analyzed 2 days after infiltration.

2.7 Confocal laser scanning microscopy analysis

The localizations of fused-proteins in *Nicotiana benthamiana* leaves were analyzed using Confocal LSM 510 META microscope. YFP fluorescence was excited with 514 nm, emission was recorded with a 530-560 nm band- pass filter, and RFP was excited with 543 nm and emission was recorded with a 560- 615 nm band- pass filter. Chlorophyll auto- fluorescence was excited with different lasers, normally 488 nm, and emission collected within 647–745 nm. Images were further analyzed using LSM 510 software and Adobe Photoshop 11.0.

3. RESULTS

3.1 Computional analysis of AtNaa60 protein sequence

In order to investigate the subcellular localization, amino acid sequence of AtNaa60 was analyzed using different tools. PredicProtein predicted two amphipathic α-helices located in the C-terminal region of AtNaa60, α-helice 1 (17 aa from 144-161) and α-helice 2 (17 aa from 205-222). The hydrophobic amino acid of α-helice 1 consists of eight residues and the hydrophobic amino acid of α-helice 2 consists of ten residues (Figure 1). These two amphipathic α-helices indicate potential interaction of AtNaa60 C-terminus with the fatty acid tails of plasma membrane or endo membrane system such as endoplasmic reticulum, Golgi apparatus and tonoplast. However, up to now AtNaa60 was only reported to be found in the plasma membrane [25], the other possibility has not been mentioned. In case of human, Naa60 also possesses 2 transmembrane α-helices in the C-terminal end but localizes in the Golgi not in the plasmamembrane [24, 26].

Normally a signal peptide is required for proteins to be transported to their appropriate destinations in the cell. Bedises two α-helice regions, AtNaa60 does not constain NLS sequence (analyzed by NLS Mapper [27]) nor transit peptides (analyzed by TargetP [28]) excluding the possibility of AtNaa60 localizes to the other organelles like nucleus, mitochondria or chloroplast.

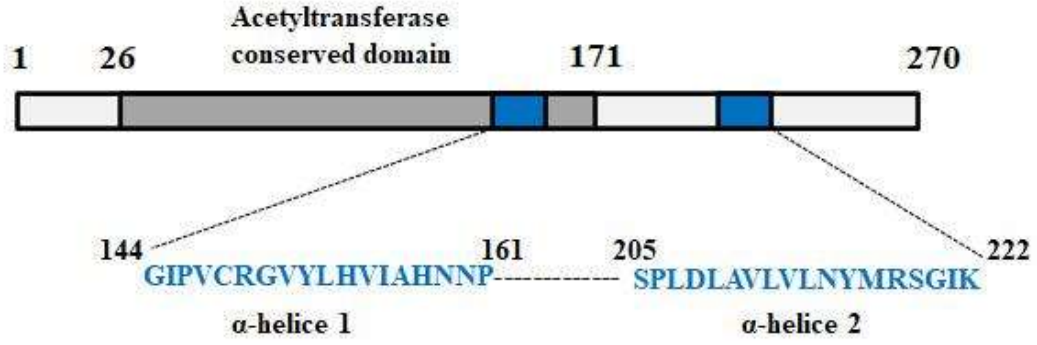

Figure 1: Analysis of AtNaa60 amino acid sequence. AtNaa60p is a protein of 270 amino acids with two transmembrane helices (144-161 and 205-222) located in the C-teminal region. The acetyltransferase conserved domain is between amino acids 26-171. The transmembrane helices were predicted by PredictProtein [29]. SMART [30] was used to analyze the acetyltransferase domain.

3.2 AtNaa60 localizes to the plasma membrane and tonoplast

In order to experimentally investigate where AtNaa60p localize, EYFP was fused to the C-terminus or N-terminus of AtNaa60p and transiently expressed in *Arabidopsis* protoplasts. *ATNAA60* cDNA was amplified by PCR with specific primers, attB1 and attB2 sequences were added into primers for cloning into Donor vector using GATEWAY system. In case of C-terminal fusion, the stop codon was eliminated

to ensure the continuos fusion with EYFP. The amplified fragments in Donor vector were confirmed by sequencing before transfer to destination vectors (pB7YWG2 and pB7WGY2).

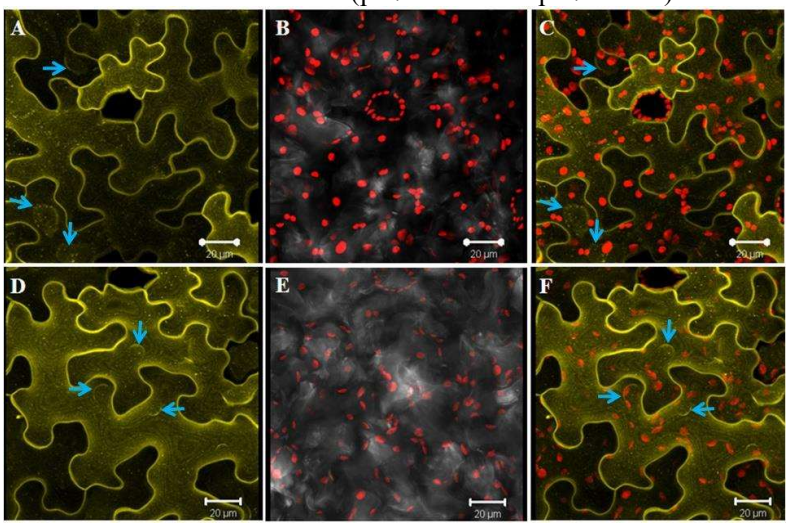

Figure 2: Transient expression of YFP-fused AtNaa60p in tobacco leaves. *Agrobacterium tumefaciens* carrying pB7YGW2-NAA60 or pB7WGY2-NAA60 was infiltrated into tobacco leaves. Two days after infiltration, the expression was analyzed using confocal microscopy. Blue arrow indicates tonoplast-like structure. (A, B, C) Expression of AtNaa60p fused with EYFP at the C-terminus. (D, E, F) Expression of AtNaa60p fused with EYFP at the N-terminus. (A and D) YFP signal, (B and E) autofluorescence of chloroplasts, (C and F) overlay.

pB7YWG2 vector (expresses AtNaa60-EYFP) or pB7WGY2 vector (expresses EYFP-AtNaa60) was infiltrated into tobacco leaf using *Agrobacterium tumefaciens* strain GV3101. The subcellular localization of AtNaa60 was shown in the Figure 2. Transient expression of AtNaa60 fused with EYFP at the C-terminus (Figure 2 A, B, C) or N-terminus (Figure 2 D, E, F) displayed the same parttern and clearly indicates the subcellular localization of AtNaa60 to the plasma membrane. The plasma membrane localization was suitable with the theoretical analysis of amino acid sequence above and was in agreement with the study of Lister and co-workers [25]. In their research, AtNaa60 was fused CFP at the C-termini and overexpressed in tobacco leaf. The CFP signal indicated the localization of AtNaa60 to the plasma membrane. YFP and CFP fusions did not affect on the sorting of AtNaa60 in the cell and strongly reinforce its location to the membrane. In addition, the same YFP signals detected in the tobacco leaves fused with pB7YWG2 or pB7WGY2 showed that different terminal fusions with EYFP did not affect on the localization of AtNaa60. Notably, a thoroughly analysis of YFP signals revealed other localization of AtNaa60 to the vacuole-like structure besides plasma membrane as seen in the Figure 3.2 (blue arrows).

To confirm the the localization of AtNaa60, pB7YWG2 was co-transfected with pKF13-TMD23-RFP which expresses RFP plasma membrane marker into tobacco leaf. As shown in the Figure 3, the YFP and RPF signals were partly overlapping. Partly overlapping signals not only confirmed the plasma membrane subcellular localization of AtNaa60 but also pointed out other membrane-like compartment location, most likely the tonoplast.

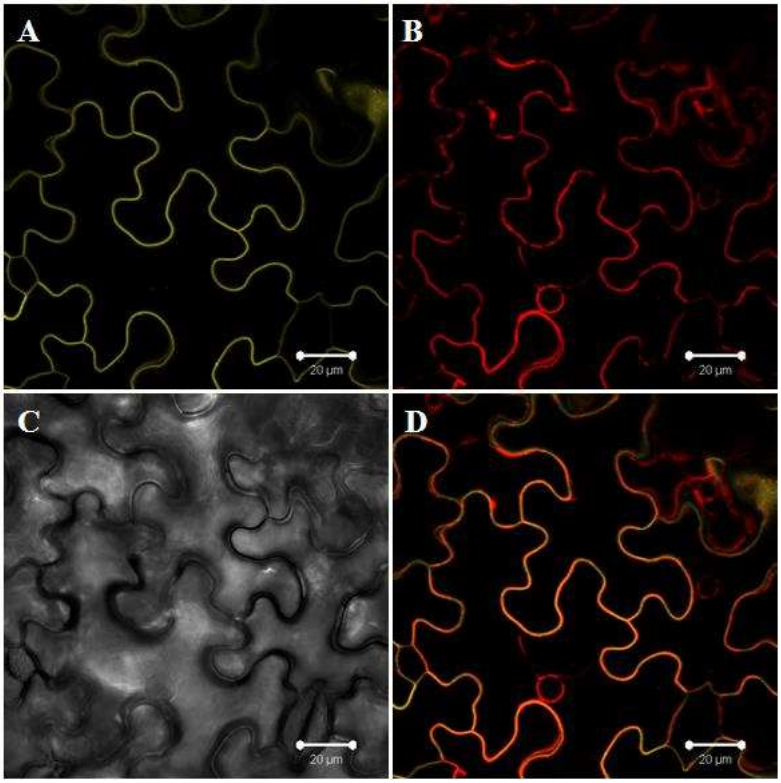

Figure 3: Expression of tobacco leaves harbouring p35S:AtNaa60-YFP and pUbi-TDM23-RFP. *Agrobacterium tumefaciens* carrying pB7YGW2-AtNaa60 (expresses AtNaa60-YFP) and *Agrobacterium tumefaciens* carrying pUbi-TDM23-RFP were mixed and co-infiltrated into tobacco leaves. Two days after infiltration, the expression was analyzed using confocal microscopy (A) YFP signal. (B) RFP signal. (C) Bright field. (D) Partly overlay of YFP signal and RFP signal indicates the localization of AtNaa60p not only to the plasma membrane but also to the other compartments.

Normally, in plant the centre vacuole occupies most of the cell space and gets close to plasma membrane and the cell wall (Figure 2 and Figure 3), therefore it interferes the localization analysis. To distinguish between the localization in plasma membrane or tonoplast, tobacco leaves were cut into small pieces (1cm x 1cm) and soaked in 0.5 M NaCl solution for 15 minutes. Under high osmotic pressure, the plasma membrane was sunk away and separated from cell wall and tonoplast (Figure 4). As shown in the Figure 4A and Figure 4D, two chloroplasts located in the interspace of two separated membranes which are the tonoplast and plasma membrane. The detection of YFP signal in these membranes (Figure 4C and Figure 4D) clearly proved the localization of AtNaa60p both to the tonoplast and to the plasma membrane. So far, of those seven NAT complexes (NatA-NatH) that have been identified in higher eukaryotes, plant NatF is the first NAT that located both in the plasma membrane and in the tonoplast. The localization of Naa60 to the plasma membrane and tonoplast is the unique characteristics of plants since they are the only ongarnisms that possess a large centre vacuole. Although the fluorescent fusion strongly indicates AtNaa60 subcellular localization also to the tonoplast, other experiments such as immunofluorescence might be needed to confirm this result since overexpression of YPF fusion or co-overexpression with other fluorescent marker could partly affect on the protein sorting process in the cell.

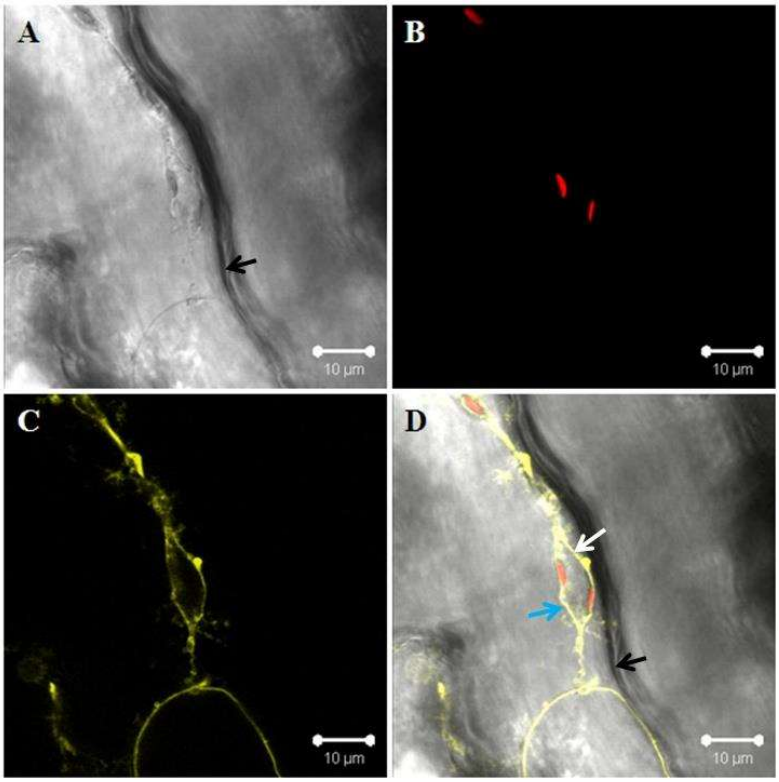

Figure 4: Expression of AtNaa60-YFP fused protein in tobacco leaves after plasmolysis. Small pieces (1cm x 1cm) of tobacco leaves expressing AtNaa60-YFP fused protein were treated with 0,5 M NaCl for 15 munutes before analyzing using confocal microscopy. Black arrow indicates cell wall, blue arrow indicates tonoplast and white arrow indicates plasma membrane. (A) Bright field. (B) Autofluorescence of chloroplasts. (C) YFP signal. (D) Overlay of A, B and C.

4 CONCLUSSIONS

Amino acid sequence analysis reveals two amphipathic α-helices located in the C-terminal region suggesting the possibility of AtNaa60 interacting with the fatty acid tails of the membrane. Transient expression of EYFP-fused AtNaa60 in tobacco leaf confirms the subcellular localization of AtNaa60 to the plasma membrane. Co-transformation with RFP membrane marker points out other localization of AtNaa60 to the tonoplast but still need further investigation.

ACKNOWLEDGMENT: This work was done at the Department of Plant Molecular Biology, University of Heidelberg, Germany. T. V. Dinh thanks the University of Heidelberg for giving an opportunity to complete this research.

REFERENCES

[1] T. Arnesen, Towards a functional understanding of protein N-terminal acetylation, *PLoS Biol*, vol. 9, e1001074, 2011.

[2] W.V. Bienvenut, D. Sumpton, A. Martinez, S. Lilla, C. Espagne, T. Meinnel, C. Giglione, Comparative large scale characterization of plant versus mammal proteins reveals similar and idiosyncratic N-alpha-acetylation features, *Mol Cell Proteomics*, vol. 11, M111 015131, 2012.

[3] M. Falb, M. Aivaliotis, C. Garcia-Rizo, B. Bisle, A. Tebbe, C. Klein, K. Konstantinidis, F. Siedler, F. Pfeiffer, D. Oesterhelt, Archaeal N-terminal protein maturation commonly involves N-terminal acetylation: a large-scale proteomics survey, *J Mol Biol*, vol. 362, pp. 915-924, 2006.

[4] J. Soppa, Protein acetylation in archaea, bacteria, and eukaryotes, *Archaea*, 2010.

[5] A.T. Coulton, D.A. East, A. Galinska-Rakoczy, W. Lehman, D.P. Mulvihill, The recruitment of acetylated and unacetylated tropomyosin to distinct actin polymers permits the discrete regulation of specific myosins in fission yeast, *J Cell Sci*, vol. 123, pp. 3235-3243, 2010.
[6] D.C. Scott, J.K. Monda, E.J. Bennett, J.W. Harper, B.A. Schulman, N-terminal acetylation acts as an avidity enhancer within an interconnected multiprotein complex, *Science*, vol. 334, pp. 674-678, 2011.
[7] G.M. Forte, M.R. Pool, C.J. Stirling, N-terminal acetylation inhibits protein targeting to the endoplasmic reticulum, *PLoS Biol*, vol. 9, e1001073, 2011.
[8] W.M. Holmes, B.K. Mannakee, R.N. Gutenkunst, T.R. Serio, Loss of amino-terminal acetylation suppresses a prion phenotype by modulating global protein folding, *Nat Commun*, vol. 5, pp. 4383, 2014.
[9] C.S. Hwang, A. Shemorry, A. Varshavsky, N-terminal acetylation of cellular proteins creates specific degradation signals, *Science*, vol. 327, pp. 973-977, 2010.
[10] T. Esmailpour, H. Riazifar, L. Liu, S. Donkervoort, V.H. Huang, S. Madaan, B.M. Shoucri, A. Busch, J. Wu, A. Towbin, R.B. Chadwick, A. Sequeira, M.P. Vawter, G. Sun, J.J. Johnston, L.G. Biesecker, R. Kawaguchi, H. Sun, V. Kimonis, T. Huang, A splice donor mutation in NAA10 results in the dysregulation of the retinoic acid signalling pathway and causes Lenz microphthalmia syndrome, *J Med Genet*, vol. 51, pp. 185-196, 2014.
[11] C. Koufaris, A. Kirmizis, N-Terminal Acetyltransferases Are Cancer-Essential Genes Prevalently Upregulated in Tumours, *Cancers (Basel)*, vol. 12, 2020.
[12] M. Chaibva, S. Jawahery, A.W.t. Pilkington, J.R. Arndt, O. Sarver, S. Valentine, S. Matysiak, J. Legleiter, Acetylation within the First 17 Residues of Huntingtin Exon 1 Alters Aggregation and Lipid Binding, *Biophys J*, vol. 111, pp. 349-362, 2016.
[13] J. Hollebeke, P. Van Damme, K. Gevaert, N-terminal acetylation and other functions of Nalpha-acetyltransferases, *Biol Chem*, vol. 393, pp. 291-298, 2012.
[14] A. Drazic, L.M. Myklebust, R. Ree, T. Arnesen, The world of protein acetylation, *Biochim Biophys Acta*, vol. 1864, pp. 1372-1401, 2016.
[15] P. Pesaresi, N.A. Gardner, S. Masiero, A. Dietzmann, L. Eichacker, R. Wickner, F. Salamini, D. Leister, Cytoplasmic N-terminal protein acetylation is required for efficient photosynthesis in Arabidopsis, *Plant Cell*, vol. 15, pp. 1817-1832, 2003.
[16] E. Linster, I. Stephan, W.V. Bienvenut, J. Maple-Grodem, L.M. Myklebust, M. Huber, M. Reichelt, C. Sticht, S.G. Moller, T. Meinnel, T. Arnesen, C. Giglione, R. Hell, M. Wirtz, Downregulation of N-terminal acetylation triggers ABA-mediated drought responses in Arabidopsis, *Nat Commun*, vol. 6, pp. 7640, 2015.
[17] M. Huber, W.V. Bienvenut, E. Linster, I. Stephan, L. Armbruster, C. Sticht, D. Layer, K. Lapouge, T. Meinnel, I. Sinning, C. Giglione, R. Hell, M. Wirtz, NatB-Mediated N-Terminal Acetylation Affects Growth and Biotic Stress Responses, *Plant Physiol*, vol. 182, pp. 792-806, 2020.
[18] P. Van Damme, K. Hole, A. Pimenta-Marques, K. Helsens, J. Vandekerckhove, R.G. Martinho, K. Gevaert, T. Arnesen, NatF contributes to an evolutionary shift in protein N-terminal acetylation and is important for normal chromosome segregation, *PLoS Genet*, vol. 7, e1002169, 2011.
[19] T. Arnesen, Protein N-terminal acetylation: NAT 2007-2008 Symposia, *BMC Proc*, vol. 3 Suppl 6, S1, 2009.
[20] H. Aksnes, P. Van Damme, M. Goris, K.K. Starheim, M. Marie, S.I. Stove, C. Hoel, T.V. Kalvik, K. Hole, N. Glomnes, C. Furnes, S. Ljostveit, M. Ziegler, M. Niere, K. Gevaert, T. Arnesen, An organellar nalpha-acetyltransferase, naa60, acetylates cytosolic N termini of transmembrane proteins and maintains Golgi integrity, *Cell Rep*, vol. 10, pp. 1362-1374, 2015.
[21] T.V. Dinh, W.V. Bienvenut, E. Linster, A. Feldman-Salit, V.A. Jung, T. Meinnel, R. Hell, C. Giglione, M. Wirtz, Molecular identification and functional characterization of the first Nalpha-acetyltransferase in plastids by global acetylome profiling, *Proteomics*, vol. 15, pp. 2426-2435, 2015.
[22] R. Ree, S. Varland, T. Arnesen, Spotlight on protein N-terminal acetylation, *Exp Mol Med*, vol. 50, pp. 1-13, 2018.
[23] K. Hole, P. Van Damme, M. Dalva, H. Aksnes, N. Glomnes, J.E. Varhaug, J.R. Lillehaug, K. Gevaert, T. Arnesen, The human N-alpha-acetyltransferase 40 (hNaa40p/hNatD) is conserved from yeast and N-terminally acetylates histones H2A and H4, *PLoS One*, vol. 6, e24713, 2011.
[24] H. Aksnes, M. Goris, O. Stromland, A. Drazic, Q. Waheed, N. Reuter, T. Arnesen, Molecular determinants of the N-terminal acetyltransferase Naa60 anchoring to the Golgi membrane, *J Biol Chem*, vol. 292, pp. 6821-6837, 2017.
[25] E. Linster, D. Layer, W.V. Bienvenut, T.V. Dinh, F.A. Weyer, W. Leemhuis, A. Brunje, M. Hoffrichter, P. Miklankova, J. Kopp, K. Lapouge, J. Sindlinger, D. Schwarzer, T. Meinnel, I. Finkemeier, C. Giglione, R. Hell, I. Sinning, M. Wirtz, The Arabidopsis N(alpha) -acetyltransferase NAA60 locates to the plasma membrane and is vital for the high salt stress response, *New Phytol*, 2020.
[26] J.Y. Chen, L. Liu, C.L. Cao, M.J. Li, K. Tan, X. Yang, C.H. Yun, Structure and function of human Naa60 (NatF), a Golgi-localized bi-functional acetyltransferase, *Sci Rep*, vol. 6, pp. 31425, 2016.

[27] S. Kosugi, M. Hasebe, M. Tomita, H. Yanagawa, Systematic identification of cell cycle-dependent yeast nucleocytoplasmic shuttling proteins by prediction of composite motifs, *Proc Natl Acad Sci U S A*, vol. 106, pp. 10171-10176, 2009.
[28] J.J. Almagro Armenteros, M. Salvatore, O. Emanuelsson, O. Winther, G. von Heijne, A. Elofsson, H. Nielsen, Detecting sequence signals in targeting peptides using deep learning, *Life Science Alliance*, vol. 2, e201900429, 2019.
[29] G. Yachdav, E. Kloppmann, L. Kajan, M. Hecht, T. Goldberg, T. Hamp, P. Honigschmid, A. Schafferhans, M. Roos, M. Bernhofer, L. Richter, H. Ashkenazy, M. Punta, A. Schlessinger, Y. Bromberg, R. Schneider, G. Vriend, C. Sander, N. Ben-Tal, B. Rost, PredictProtein--an open resource for online prediction of protein structural and functional features, *Nucleic Acids Res*, vol. 42, pp. W337-343, 2014.
[30] I. Letunic, T. Doerks, P. Bork, SMART 7: recent updates to the protein domain annotation resource, *Nucleic Acids Res*, vol. 40, pp. D302-305, 2012.

XÁC ĐỊNH SỰ CƯ TRÚ TRONG TẾ BÀO CỦA ARABIDOPSIS N^{α}-ACETYLTRANSFERASE 60 BẰNG BIỂU HIỆN TẠM THỜI TRONG LÁ CÂY THUỐC LÁ

Tóm tắt. Acetyl hóa đầu N của Protein (NTA) xúc tác bởi Nα-acetyltransferases (NAT) là một trong những biến đổi phổ biến nhất ở sinh nhật nhân chuẩn. NTA có vai trò quan trọng trong tính ổn định, sự liên kết và quá trình vận chuyển protein. Gần đây Nα-acetyltransferase 60 (Naa60/NatF) đã được nhận diện ở sinh vật bậc cao. Ở người, Naa60 gắn với thể Golgi và chịu trách nhiệm trong việc NTA protein tế bào chất và protein màng. Tuy nhiên ở thực vật, sự cư trú của Naa60 trong tế bào chưa được đề cập tới một cách đúng mức. Nghiên cứu này tập trung nghiên cứu sự cư trú của Arabidopsis Naa60 trong tế bào. Phân tích trình tự amino acid bằng phần mềm PredictProtein cho thấy Naa60 có khả năng cư trú trên màng tế bào với hai chuỗi xoắn lưỡng tính α-helice tại đầu C. Biểu hiện tạm thời *Arabidopsis* Naa60 gắn với EYFP tại đầu N- và C- ở lá cây thuốc lá khẳng định sự cư trú của Naa60 trên màng tế bào. Đồng biểu hiện với RFP marker cho thấy Naa60 còn cư trú trên màng không bào

Từ khóa. N^{α}-acetyltransferase, *Arabidopsis thaliana*, Naa60, cư trú trong tế bào, xâm nhập qua lá

Received on: 02/08/2021
Accepted on: 22/11/2021