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Lysine 88 Acetylation Negatively Regulates Ornithine Carbamoyltransferase Activity in Response to Nutrient Signals

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Ornithine carbamoyltransferase (OTC) is a key enzyme in the urea cycle to detoxify ammonium produced from amino acids catabolism. OTC deficiency is an X-linked genetic disorder ranging from fatal in newborns to hyperammonemia and anorexia in adults. Through affinity purification of acetylated peptides and mass spectrometry, we identified that OTC is acetylated on lysine residues, including K88, which is also mutated in OTC deficiency patients. OTC acetylation was confirmed to occur under physiological conditions. Biochemical characterizations revealed that OTC K88 acetylation decreases the affinity for carbamoyl phosphate, one of the two OTC substrates, and the maximum velocity while the K_m for ornithine, the other OTC substrate, is not affected. Furthermore, K88 acetylation is regulated by both extracellular glucose and amino acid availability, indicating that OTC activity may be regulated by cellular metabolic status. Our results provide an example of novel mechanism of regulating metabolic enzyme activity through protein acetylation.

Ornithine carbamoyltransferase is an X-linked mitochondrial enzyme mainly expressed in hepatocytes and enterocytes. OTC is synthesized as a precursor and the mitochondrial import is accompanied by removal of the N terminal signal peptide to produce a 36 kDa mature enzyme. As a key urea cycle enzyme, OTC catalyzes the reaction that converts ornithine and carbamoyl phosphate into citrulline. Ornithine is the deamination product of arginine while carbamoyl phosphate is the condensation product of ammonium generated by amino acid deamination and carbon dioxide. Thus, OTC is of crucial importance for cellular ammonium secretion (as a form of urea) and amino acid catabolism (1).

Because there is no alternative way of urea synthesis, blockade in the urea cycle results in devastating health consequences. OTC mutation is a relative common genetic disorder in human. A deficiency of OTC usually results in central nervous system dysfunction, which may cause irreversible brain damage or be fatal in newborn infants (2). OTC deficiency in adults has milder symptoms but still cause health problems such as hyperammonemia (3,4) and anorexia(5). Because the gene is on X-chromosome, OTC deficiency is much more prevalent in males. There are more than 340 OTC mutations identified so far with clinical symptoms (6). No obvious hotspot for OTC mutations has been found. All null mutations are associated with severe neonatal phenotypes and none survived beyond five days of life without treatment. Most disease associated OTC mutants are missense mutations(7). These observations suggest an indispensable role for OTC activity in human physiology.

Addition of acetyl moiety to the ε-amino group of a lysine residue is a common mechanism of posttranslational modification. Protein acetylation regulates various aspects of protein functions, such as regulating protein-protein interaction (8). The role of acetylation in regulating nuclear proteins and transcriptional factors has been well established after more than a decade of intense research. Histone acetylation affects chromatin structure and gene expression (9-11); tumor suppressor protein p53 can be acetylated at multiple sites, and different acetylation modifications have distinct effects on p53 function

and physiological impact on the cell (12,13). The regulatory role of acetylation on enzyme activity was first reported in acetyl-CoA synthetase(14), in which the active site lysine residue in the *Salmonella Enterica* enzyme is acetylated and therefore acetylation inhibits acetyl-CoA synthetase activity. Acetyl-CoA synthetase in other species has also been reported to be regulated by acetylation of the active site lysine residue (15,16), indicating a conserved regulatory mechanism by acetylation for this enzyme.

We performed a proteomic analysis of protein acetylation in human liver. Our studies have identified that lysine residue 88 in OTC is acetylated. Interestingly, OTC three dimensional structure (17-19) shows that K88 is not only localized near the carbamoyl phosphate binding residues (residue 90 to 93), but also involved in the formation of a complex hydrogen-bonded network that directly participates in substrate binding, indicating a critical role of K88 in catalysis. Furthermore, mutation of K88 is found in human OTC deficient patients (20). These two lines of evidence suggest that acetylation on K88 may play a key role in the regulation of OTC activity. In this study, we have characterized the OTC acetylation and found that K88 acetylation inhibits OTC activity. Furthermore, the K88 acetylation is affected by cellular metabolic status, indicating a possible role of acetylation in physiological regulation.

EXPERIMENTAL PROCEDURES

Cell Culture and transfection- HEK293T and Chang's liver cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) supplemented with 10% fetal calf serum (HyClone), 100 U/ml penicillin and streptomycin (Gibco). Cell transfection was performed using Lipofectamine 2000 (Invitrogen) or calcium phosphate methods.

Polyclonal antibody generation- Pan-acetyllysine polyclonal antibodies were generated using chemically modified acetylated chicken ovalbumin as antigen. Polyclonal antibodies against acetylated OTC K88 were generated in rabbits using an acetylated peptide (GMIFEK[Ac]RSTRT) as antigen.

Deacetylase inhibitors treatment- Deacetylase inhibitor treatments were carried out by adding trichostatin A (TSA, 0.5μM) and/or nicotinamide (NAM, 5 mM) into culture medium 16 h before harvesting; both concentrations are final concentrations in culture medium.

Glucose/amino acids treatment- Cells or transfected cells were kept in DMEM medium for 24 h before treatment. Cells were washed twice with PBS buffer and continued culture in Dulbecco's Modified Eagle's Medium (SIGMA, D5030, without glucose but with essential amino acids) based medium with desired supplementation of glucose or amino acids. Amino acids concentrations were the total concentrations of equal molar of glutamate and aspartate. Cells were harvested after 12 h of treatment.

Immunoprecipitation- Cells were lysed with lysis buffer (50 mM Tris • HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 1% NP-40, 50 mM NaF, 1.5 mM Na_3VO_4, protease inhibitor cocktail [Roche], 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). For anti-FLAG IP, 500 μl of cell lysate was incubated with anti-FLAG M2-agarose for 4 h at 4°C; for OTC antibody IP, lysate was incubated with anti-OTC antibody(Aviva Systems Biology, 1:500) overnight and then protein A/G beads were added and incubation continued for another 2 h. Beads were washed three times with lysis buffer, and the FLAG tagged proteins were eluted by FLAG peptides (Gilson Biochemical).

His tagged Protein expression and Purification- His tagged OTC wild type, K88Q and K88R proteins were expressed in *Escherichia coli*, BL21(DE3). After reached a middle exponential growing stage, 0.2 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to bacterial culture to induce protein overexpression. Induction was carried out at 20°C for 4 h. Expressed proteins were purified with nickel beads (GE, Affinity Chromatography Methods Handbook). Purified proteins were stored at -80 °C in 10% glycerol.

OTC assay- OTC enzyme assay was adapted from published method (21). Briefly, 5 μl of FLAG peptides eluted ectopic OTC-FLAG solution or 10 μl of OTC antibody IPed beads was added to a solution containing ornithine and triethanolamine to a final volume of 675 μl. OTC reactions were started by adding 75μl of 150 mM carbamoyl phosphate. Final concentrations in assay of each reagent were: 5 mM ornithine, 15 mM carbamoyl phosphate, and 270 mM triethanolamine (pH 7.7). After 30 min of incubation at 37°C, reactions were stopped by adding 375 μl phosphoric acid/sulfuric acid (3:1, v/v). Citrulline production was determined by adding 47 μl of 3% 2, 3 - butanedionemonoxime, boiling in the dark for 15 min, and reading absorbance at 490 nm.

Results

OTC is acetylated at K88- Most reported acetylation studies are with nuclear proteins while few cytoplasmic protein acetylation studies have been documented. To investigate non-nuclear protein acetylation, human liver tissue was fractionated into nuclear, cytosolic, mitochondrial, and membrane fractions. The subcellular fractions were digested with trypsin and acetylated peptides were purified by anti-acetyllysine antibody followed by LC/LC-MS/MS analysis. Among many acetylated peptides identified, a peptide SLGMIFEK*R was identified in both mitochondria and membrane fraction from liver samples (Fig. 1A). Sequest search indicates that this peptide matches to human OTC protein and the acetylated lysine is K88. This acetylation peptide was identified in multiple independent experiments. Interestingly, an independent proteomic survey by Kim *et al.* also reported that the same peptide is acetylated in mouse OTC (22). K88 in OTC is highly conserved in different species from *S. cerevisiae* to human (Fig. 1B), indicating a critical role in OTC function.

We are aware of the possibility that lysine acetylation may come from the in vitro manipulation during sample preparation. For example, formic acid used in solubilizing peptides during MS assay could be contaminated by trace amount of acetate anhydrate and this contamination could cause acetylation of peptides during sample preparation. Trichostatin A (TSA) is a potent inhibitor towards both class I and class II deacetylase while nicotinamide (NAM) can inhibit class III deacetylase (23-25). To confirm that OTC K88 is acetylated *in vivo*, human OTC gene with a FLAG tag at the C-terminus was cloned into pcDNA3 and expressed in HEK293T cells and the transfected cells were treated with deacetylase inhibitors. p53, a well known acetylated protein, was included as a positive control. OTC and p53 proteins were immunoprecipitated from transfected cells followed by Western blotting for lysine acetylation. Interestingly, like p53, TSA and NAM treatment also significantly enhanced OTC acetylation (Fig. 1C). These results confirmed that OTC is acetylated *in vivo*.

To further examine if endogenous OTC is acetylated, Chang's liver cells were treated with or without the TSA plus NAM. Endogenous OTC protein was precipitated with an anti-OTC antibody and the acetylation level of OTC was probed by anti-acetyllysine antibody (α-AcK). We found that endogenous OTC acetylation level was significantly increased by treatment with TSA and NAM (Fig. 1D). These results indicate that OTC is acetylated under physiological condition and the acetylation of OTC is negatively regulated by deacetylases.

Our mass spectrometry data showed that OTC is possibly acetylated on two additional lysine residues, K46 and K231. A rise in total acetylation detected by α-AcK is not sufficient to conclude that OTC K88 is acetylated. To directly detect OTC K88 acetylation, we raised antibody against an acetylated K88 OTC peptide. As shown in Fig. 1E, the site specific antibody recognized the overexpressed wild type OTC K88 acetylation and the acetylation level of OTC K88 increased upon TSA plus NAM treatment. The antibody, however, couldn't recognize OTC K88Q mutant, indicating that the antibody is specific for acetylated K88 in OTC. The above data demonstrate that K88 in OTC is indeed acetylated.

Inhibition of deacetylase activity reduces OTC activity- Lysine 88 has been implicated to be important for OTC catalysis (26). Therefore, acetylation of K88 will likely affect its enzymatic activity. We hypothesize that if OTC K88 acetylation regulates its enzyme activity, inhibiting deacetylases could increase the acetylation level of K88 and in turn, affect OTC activity. Both endogenous OTC and overexpressed OTC activities were examined to test this hypothesis. We first examined OTC activity regulation by deacetylase inhibitors in transfected HEK293 cells. OTC was transfected into HEK293T cells and the cells were treated with NAM or TSA. OTC protein was immunopurified and activity was measured. We observed that the immunoprecipitated OTC activity was inhibited in cells treated with TSA and NAM (Fig. 2A). An inverse correlation between OTC acetylation and activity strongly indicate that OTC activity is negatively regulated by acetylation. Furthermore, Chang's liver cells were cultured in the absence or presence of deacetylase inhibitors, TSA and NAM. Endogenous OTC was IP-ed by OTC antibody conjugated protein A/G beads and OTC activity was measured on beads. We found that either TSA or NAM treatment decreased endogenous OTC activity (Fig. 2B). The reduction of OTC activity by TSA and NAM indicates that endogenous OTC activity is inhibited by acetylation.

K88 acetylation inhibits OTC activity- The human disease associated with K88N mutation

causes OTC deficiency although this mutant OTC has residual activity (27). This is consistent with our finding that inhibition of deacetylases decreases OTC activity. To test the importance of K88 acetylation in OTC regulation, we generated K88R and K88Q mutants. K88R mutation retains a positive charge and is thus considered as a conserved substitution. K88Q, on the other hand, abolishes the positive charge and therefore may mimic the effect of acetylation. Both mutants were expressed in HEK293T cells and OTC activities were determined from immunoprecipitated protein. K88R mutant retains substantial enzymatic activity although it is reduced (Fig. 3A). In contrast, K88Q mutant is essentially inactive, less than 1% of wild type OTC's activity. These results indicate that a positive charge at position 88 is critical for OTC catalytic activity. Substitution K88 by a glutamine effectively abolishes OTC activity, consistent with our notion that acetylation inhibits OTC activity. We also mutated K46 and K231, the other two acetylated lysine residues identified in our ms data, to glutamines and found that mutation of these two lysine residues had little effect on OTC activity. These results indicate that acetylation of K46 and K231 is not directly involved in enzyme activity regulation.

It is thus of interest to determine whether K88 is the primary regulatory acetylation site for OTC activity. We took the advantage of K88R mutant that retains substantial activity. We compared the enzymatic activity of wild type and K88R mutant in response to deacetylase inhibitors. As expected, treatment with TSA and NAM decreased OTC enzymatic activity with a concomitant increase in overall OTC acetylation and K88 acetylation (Fig..3B). In contrast, TSA and NAM failed to inhibit the OTC K88R mutant although overall acetylation was still increased by the deacetylase inhibitors (Fig..3B). The increase in OTC K88R acetylation is likely due to acetylation of other lysine residues in OTC. Our data strongly indicate that acetylation of K88 is responsible for OTC activity inhibition in response to deacetylase inhibitors.

The three dimensional structure of OTC suggests that a modification at K88 may affect OTC substrate binding thus affect OTC activity. To further investigate the mechanism why K88 acetylation reduces OTC activity, we expressed and purified OTC wild type, K88R and K88Q mutants from *E. coli*. Kinetic studies of the purified OTC show that substitution of K88 by either arginine or glutamine does not significantly alter the K_m for ornithine, suggesting that K88 is not directly involved in ornithine binding (Table 1). In contrast, K88Q mutant increased the K_m of carbamoyl phosphate 10 times over the wild type protein while the K88R mutant did not significantly increase the K_m. These data suggest that the positive charge residue at position 88 is important for carbamoyl phosphate binding. The above observations indicate that acetylation of K88 in OTC may decrease its substrate binding towards carbamoyl phosphate by neutralizing the positive charge of K88. Given the physiological concentration of carbamoyl phosphate is around 0.1 mM in liver cells (28,29), the acetylated OTC would have very low activity. It is worth noting that K88Q mutant not only altered OTC substrate binding but also dramatically decreased the maximum velocity. OTC K88R mutant also shows a decreased V_{max} although much less severe than the K88Q mutant. Our results support a model that OTC activity is negatively regulated by K88 acetylation.

K88 acetylation is influenced by glucose and amino acid availability- OTC functions in urea cycle and amino acid catabolism, which may be affected by the availability of cellular fuel. We therefore tested the effect of glucose and amino acids on OTC acetylation. We found that lowering the glucose levels lead to a decrease in K88 acetylation as determined by the K88 acetylation specific antibody (Fig. 4A). At the same time, OTC activity was increased by lowering glucose levels in cell culture medium (Fig. 4A), consistent with an inhibitory effect of K88 acetylation on OTC activity. This result suggests that an increase in K88 acetylation may contribute to OTC inhibition by high glucose. We also tested the effect of amino acids (glutamate and aspartate) on OTC acetylation. Surprisingly, higher amino acid concentrations also increased OTC K88 acetylation and decreased OTC activity (Fig..4B). These observations established a direct link between OTC K88 acetylation and the availability of extracellular fuels/nutrients, consistent with the notion that OTC acetylation plays a role in cellular metabolic regulation.

Discussion

The regulatory role of protein acetylation in gene expression has been well established (30). Interestingly, many metabolic enzymes are also found to be acetylated by proteomic survey in multiple species ranging from bacteria (31) to human (our unpublished data). However, the functional significance of acetylation in metabolic enzymes is largely unknown. Besides the mass

spectrometry data of identifying acetylation, few metabolic enzymes have been functionally characterized regarding the role of acetylation in physiological regulation. One example is the acetyl-CoA synthetase, which is inhibited by lysine acetylation (15). This report on OTC provides another example that acetylation inhibits the urea cycle enzyme OTC activity by modifying a critical lysine residue in substrate binding and catalysis. Moreover, our data indicate that OTC acetylation is regulated by cellular nutrient signals as OTC acetylation is enhanced by high glucose or amino acids.

Lysine 88 is the key acetylation site responsible for OTC inactivation. K88 locates in a position important for substrate binding/catalysis. Consistently, the K88Q has a much weaker affinity towards the substrate carbamoyl phosphate and is almost completely inactive (less than 1% of the wild type activity). These observations support that acetylation may inhibit OTC activity. On the other hand, K88R mutant had no effect on substrate binding and still retained approximately 20% catalytic activity. The moderate reduction in OTC activity of K88R mutation could be due to the size change of side chain by arginine substitution although arginine residue retains the positive charge. Together of the mutation analysis and acetylation study, we conclude that acetylation of K88 inhibits OTC catalytic activity. It is worth noting that mutation of K88 has been found in human OTC deficiency disease, further supporting an important role of K88 acetylation in physiological regulation.

Cells utilize glucose as the preferred energy source. When glucose is not abundant, cells shift to alternative energy sources, such as fatty acids and amino acids. Utilization of amino acids as energy source brings two problems to the cell. First, amino acids are important building blocks for proteins, biosynthesis of amino acids is energy expensive thus amino acids would not be the preferred energy source; second, amino acid catabolism produce ammonium, which is toxic to the body. Therefore, cells must adapt to metabolic pathways to get rid of ammonium. Urea cycle is a key pathway for ammonium metabolism. For these reasons, urea cycle enzyme activities need to adapt to different extracellular fuel availability. We found that when glucose concentration is high, OTC K88 acetylation is increased and the OTC activity is turned down (Fig. 4A). Therefore, the urea cycle activity is repressed in the abundance of glucose. This makes physiological sense because cells don't need to catabolize amino acids for energy in the presence of glucose. When glucose concentration is decreased, the increase of OTC activity is possibly due to K88 deacetylation. This can be explained that when cells experience glucose shortage, they need to find alternative energy source, such as amino acids. The detoxification of ammonium generated from amino acid catabolism requires high urea cycle activity. We also observed that inhibition of glycolysis by 2-deoxyglucose inhibited OTC acetylation and concomitantly increased OTC activity (data not shown). Therefore, OTC activity may be regulated by cellular energy status.

OTC K88 acetylation is also enhanced by amino acids (Fig 4B). Consistently, OTC activity is inhibited by high amino acids. However, one might expect that high amino acids should increase OTC activity due to an elevated demand for the urea cycle. Further studies are needed to clarify the physiological regulation of OTC acetylation in response to amino acids. Important future questions also include how glucose regulates OTC acetylation. We speculate that glucose may regulate OTC acetylation by affecting Sirt family deacetylase. It is possible that high glucose decreases cellular concentration of NAD, which is a co-factor for Sirt, therefore, results in a lower Sirt activity and increased OTC acetylation. In summary, our study show that K88 acetylation inhibits OTC enzymatic activity and this acetylation is regulated by cellular metabolic status and availability of nutrients.

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FOOTNOTES

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The abbreviations used are: OTC, ornithine carbamoyltransferase ; NAM, nicotinamide ; TSA trichostatin A ; IP: immunoprecipitation; K88: lysine 88; α-AcK: anti-acetyllysine antibody, PBS, phosphate-buffered saline ; IPTG, isopropyl-1-thio-β-D-galactopyranoside ; LC/LC-MS/MS, tandem liquid chromatography-mass spectrometry /mass spectrometry.

FIGURE LEGENDS

Fig 1. OTC is acetylated at Lys 88 *A.* MS/MS spectrum of acetylated OTC lysine 88 containing peptide: SLGMIFEK*R. *B.* The acetylated K88 in OTC is conserved. Sequences around OTC K88 from different species were aligned. Conserved lysine residues corresponding to human OTC K88 are boxed. *C.* Transfected OTC is acetylated. HEK293T cells were transfected with pcDNA3 vector，pcDNA3-hOTC-FLAG and pcDNA3-FLAG-p53 followed by deacetylase inhibitor treatment. Cell lysate was immunoprecipitated with FLAG beads. The precipitated OTC-FLAG was detected by FLAG and acetyllysine (α-AcK) antibody as indicated. NAM and TSA denote treatment with nicotinamide and triochostatin A, respectively. *D.* Endogenous OTC is acetylated. Chang's liver cells were treated with deacetylase inhibitors. Endogenous OTC protein was immunoprecipitated with anti-OTC antibody. The acetylation level of endogenous OTC was probed by α-AcK. ***E.*** OTC is acetylated on K88. HEK293T cells were co-transfected by pcDNA3-hOTC-FLAG and pcDNA3-hOTCK88Q-FLAG. OTC proteins were precipitated by FLAG beads. OTC K88 acetylation levels were detected by an antibody raised against OTC K88 peptide.

Fig 2. Inhibition of deacetylase reduces OTC activity. *A.* Deacetylase inhibitors decrease transfected OTC activity. HEK293T cells were transfected with pcDNA3-hOTC-FLAG and treated with deacetylase inhibitors as indicated. OTC proteins were immunoprecipitated with FLAG beads and were eluted by 100 μl FLAG peptide. OTC assay was carried out and specific activity was normalized by OTC protein levels determined by Western blot. Shown are mean value +/- standard deviation (SD) of duplicates assays. Overall acetylation level and K88 acetylation level were assayed by α-AcK or anti-acetyl-lysine88 antibody. *B.* NAM and TSA inhibit endogenous OTC activity. Chang's liver cells were treated with deacetylase inhibitors. OTC protein was immunoprecipitated with anti-OTC antibody and measure OTC activity in protein A/G beads. α-HA antibody was used as IP control. Bars and error bars represent mean +/- SD of triplicate assays. Specific OTC activities were normalized by

OTC protein level.

Fig 3. Acetylation of Lys88 inhibits OTC activity. *A.* K88 is important for full OTC activity. OTC wild type, K88R and K88Q mutants were expressed in HEK293T cells. Proteins were purified by IP and OTC activity assays were determined. Relative specific OTC activities were normalized by protein level. Wild type OTC activity was arbitrarily set as 100%. α-HA antibody was used as IP control. Bars and error bars represent mean +/- SD of triplicate assays. *B.* K88 is required for NAM and TSA to repress OTC activity. OTC and K88R mutant proteins were overexpressed in HEK293T cells followed by deacetylase inhibitor treatment. Proteins were purified by IP, total acetylation level, K88 acetylation level, and OTC activities were determined, respectively. Relative specific OTC activities were normalized by protein level. α-HA antibody was used as IP control. Bars and error bars represent mean +/- SD of triplicate assays. *C.* Kinetic analysis of OTC K88R and K88Q mutants.(Table1) OTC protein was expressed and purified from E. coli. OTC activity kinetic parameters presented as mean +/- SD. Mean values are taken from duplicate assays.

Fig 4. K88 acetylation is influenced by glucose and amino acid availability. *A.* Glucose increases OTC acetylation and decreases activity. OTC-FLAG proteins were overexpressed in HEK293T cells cultured under different glucose concentrations as indicated. Proteins were purified by IP. OTC activity, OTC acetylation and K88 acetylation were each carried out for purified proteins. Mean +/- SD values of triplicate assays for relative OTC activities were presented. Enzyme activity under 0 mM amino acids condition was arbitrarily set as 100%. All specific activities were normalized by protein level. *B.* Amino acids increases OTC K88 acetylation and decreases activity. OTC-FLAG proteins were overexpressed in HEK293T cells cultured under different amino acids concentrations as indicated. Proteins were purified by IP. OTC activity, OTC acetylation and K88 acetylation were each carried out for purified proteins. Mean +/- SD values of triplicate assays for relative OTC activities were presented. Enzyme activity under 25 mM glucose condition was arbitrarily set as 100%. All specific activities were normalized by protein levels.

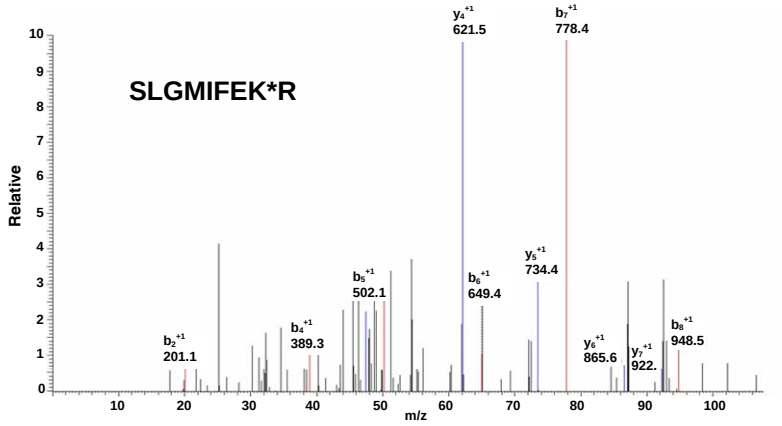
Table 1

Kinetic parameters for OTC K88R and K88Q mutants

Enzyme	*Vmax*(umol·mg-1·min-1)	*Km*-CP(mM)	*Km*-Ornithine(mM)
Wild OTC	90.9±3.5	0.13±0.01	0.36±0.13
OTC K88Q	0.6±0.1	1.24±0.20	0.55±0.08
OTC K88R	16.7±1.9	0.15±0.03	0.42±0.09

Figure 1

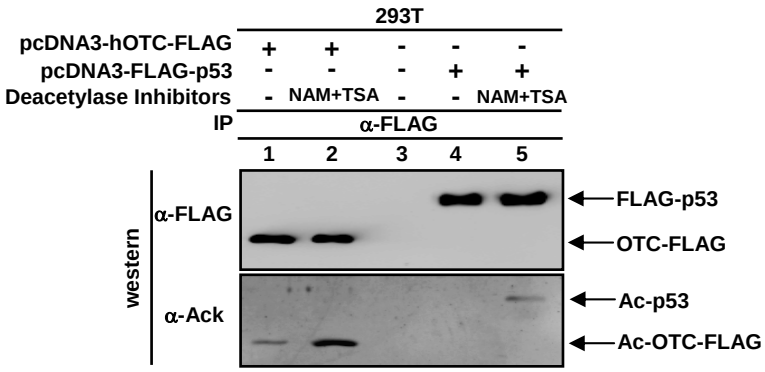
A



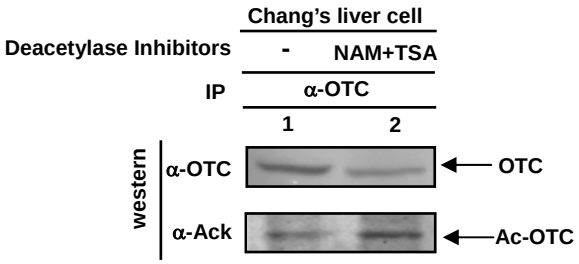
B

H.sapiens	OTC:	78	QGKSLGMIFEKRSTRTRLSTE	98
M.musculus	OTC:	78	QGKSLGMIFEKRSTRTRLSTE	98
X.laevis	OTC:	78	QGKSLAMIFEKRSIRTRLSNE	98
C.elegans	OTC:	78	QGKSLAMIFEKRSTRTRLSTE	98
A.thaliana	OTC:	111	KGKSMSMIFAKPSMRTRVSFE	131
S.pombe	ARG3:	51	SSQNVAMIFSKRSTRTRVSVE	71
S.cerevisiae	ARG3:	55	LGRTIALIFTKRSTRTRISTE	75

C



D



E

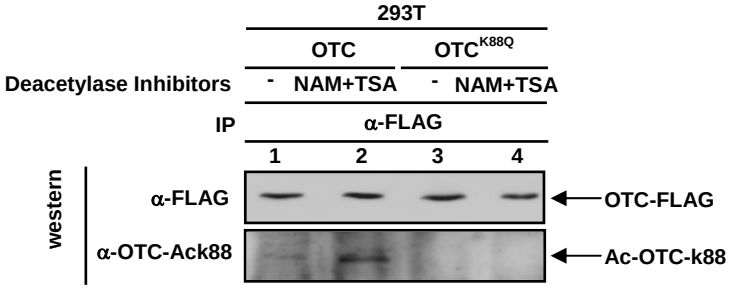


Figure 2

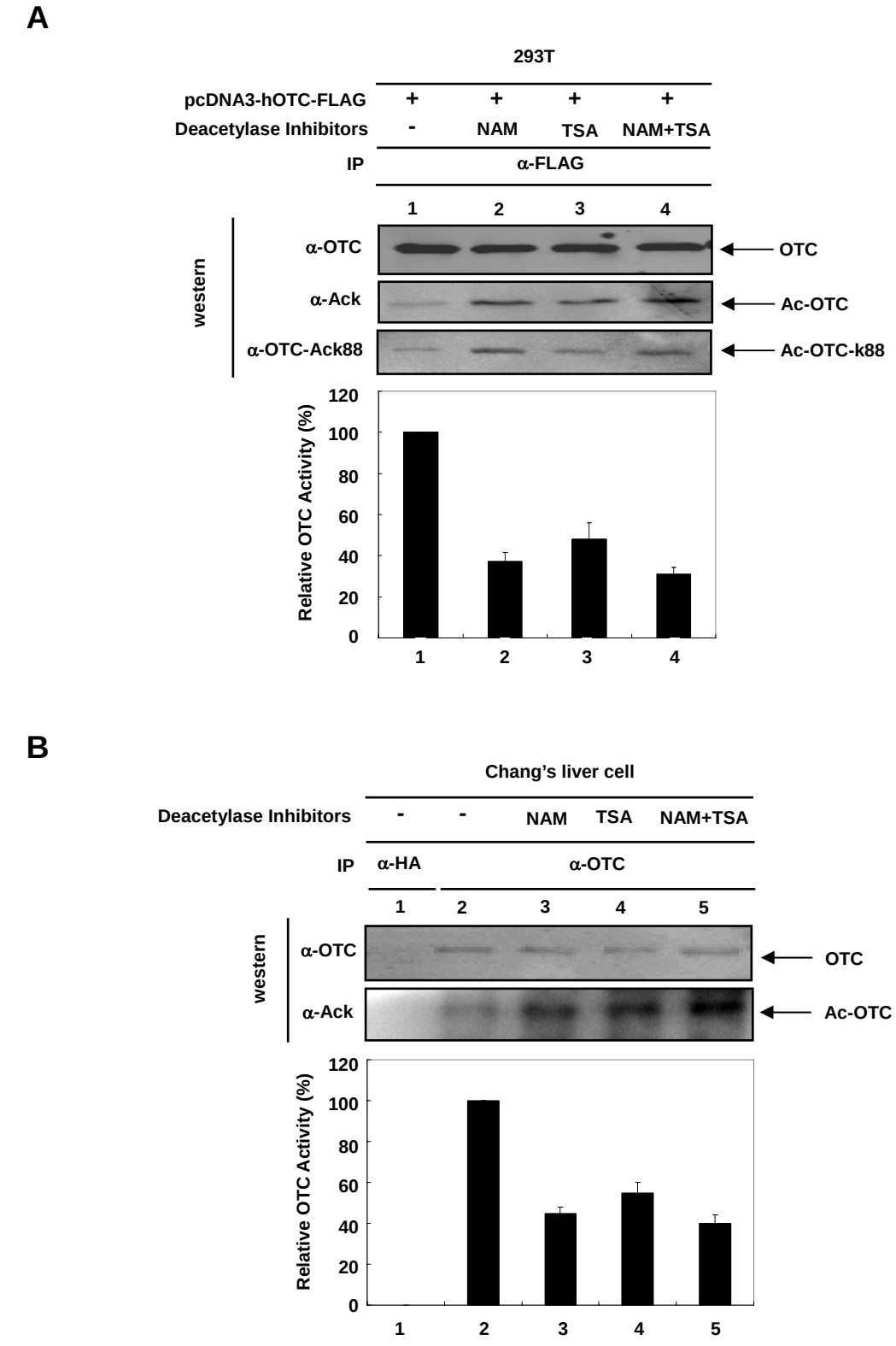


Figure 3

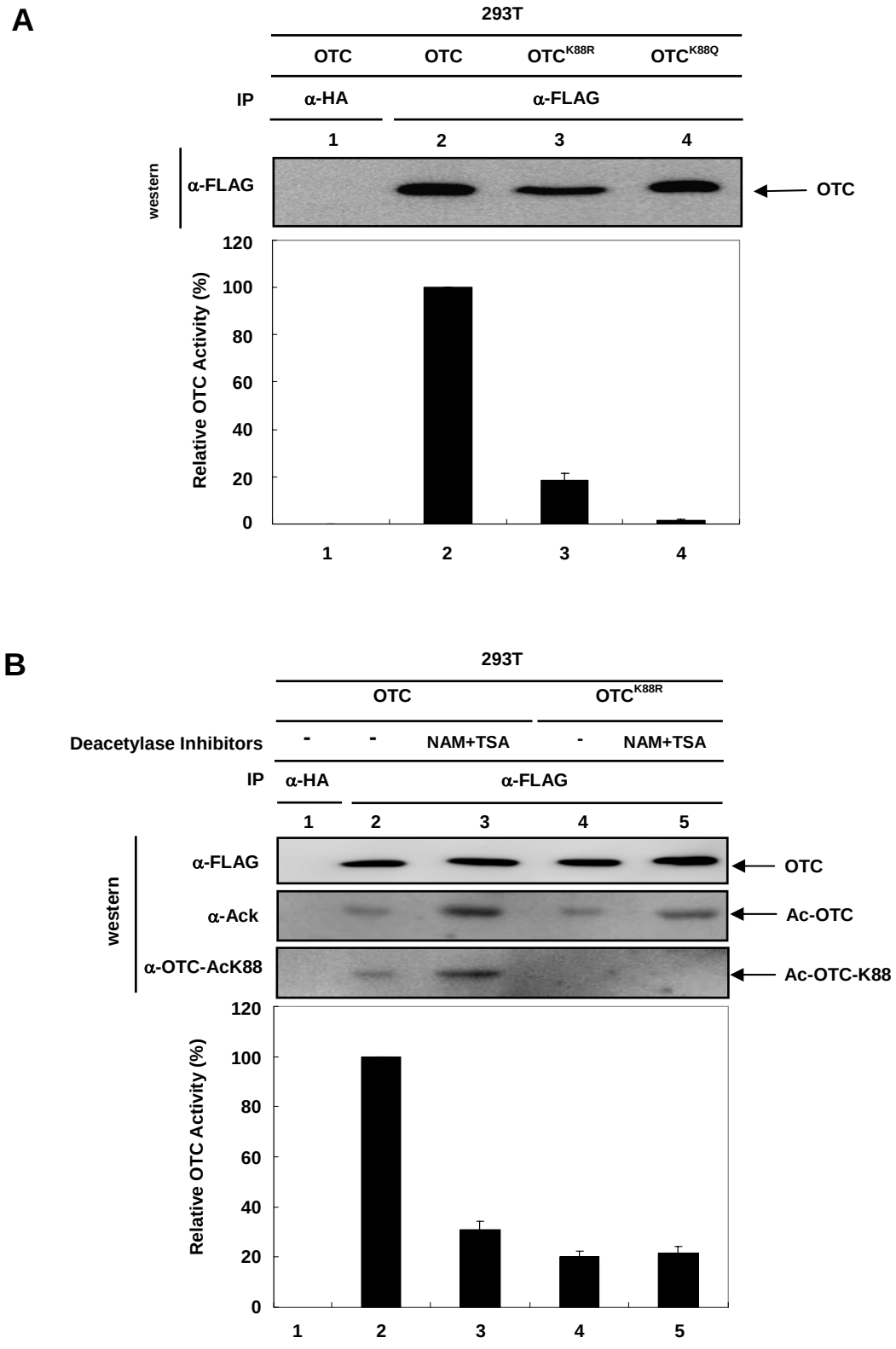
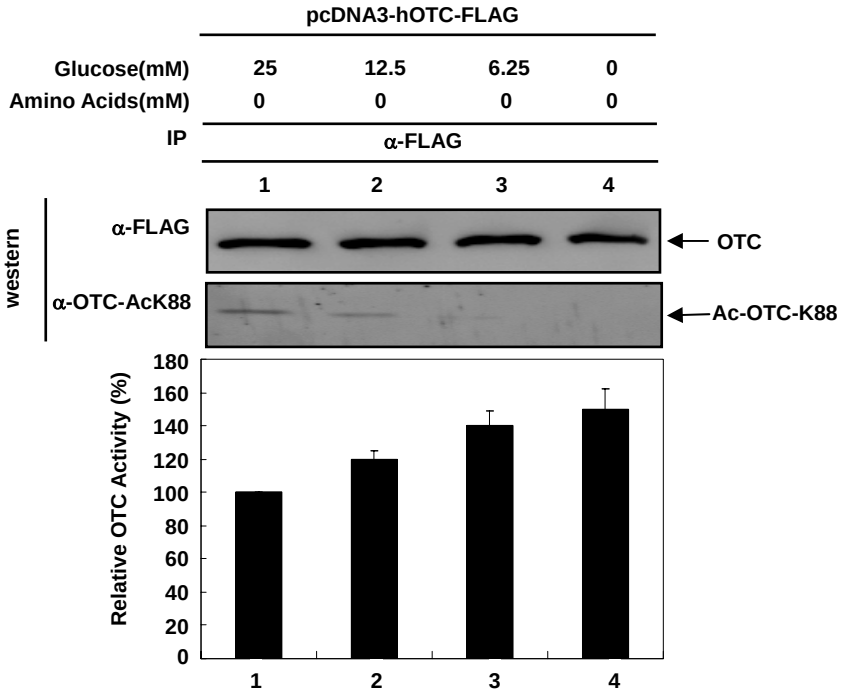
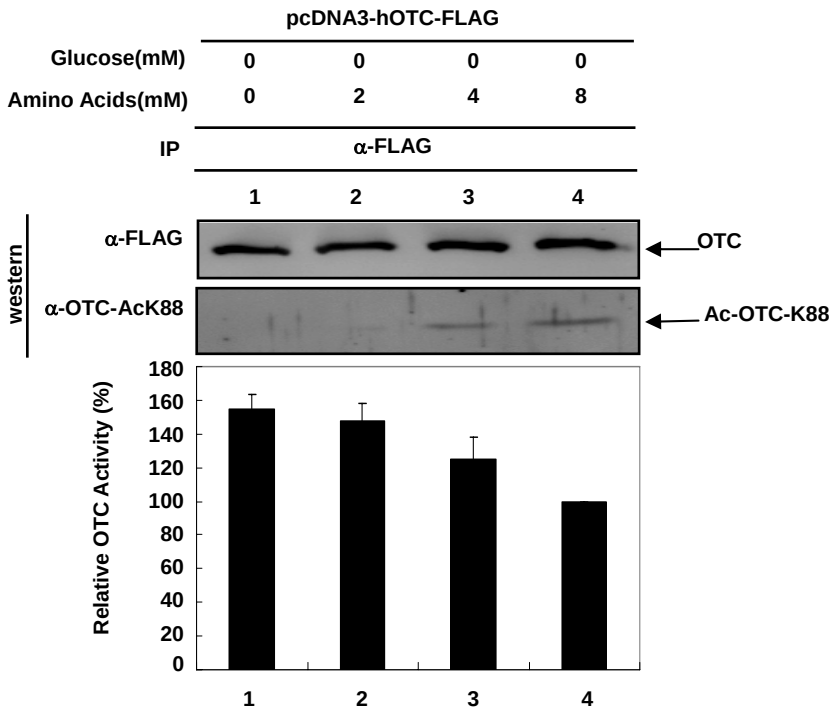


Figure 4

A



B



Lysine 88 acetylation negatively regulates ornithine carbamoyltransferase activity in response tonutrient signals

Wei Yu, Yan Lin, Jun Yao, Wei Huang, Qunying Lei, Yue Xiong, Shimin Zhao and Kun-Liang Guan

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