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Sp1 modulates ncOGT activity to alter target recognition and enhanced thermotolerance in *E. coli*

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ABSTRACT

cDNAs encoding three isoforms of OGT (ncOGT, mOGT, and sOGT) were expressed in *Escherichia coli* in which the coexpression system of OGT with target substrates was established in vivo. No endogenous bacterial proteins were significantly *O*-GlcNAcylated by any type of OGT isoform while co-expressed p62 and Sp1 were strongly *O*-GlcNAcylated by ncOGT. These results suggest that most of bacterial proteins appear not to be recognized as right substrates by mammalian OGT whereas cytosolic environments may supply UDP-GlcNAc enough to proceed to *O*-GlcNAcylation in *E. coli*. Under these conditions, sOGT was auto-*O*-GlcNAcylated whereas ncOGT and mOGT were not. Importantly, we found that when Sp1 was coexpressed, ncOGT can *O*-GlcNAcylate not only Sp1 but also many bacterial proteins. Our findings suggest that Sp1 may modulate the capability of target recognition of ncOGT by which ncOGT can be led to newly recognize bacterial proteins as target substrates, finally generating the *O*-glyco-bacteria. Our results demonstrate that the *O*-glyco-bacteria showed enhanced thermal resistance to allow cell survival at a temperature as high as 52 °C.

Addition of *N*-acetylglucosamine (GlcNAc) through β-*O*-glycosidic linkage to serine/threonine residues on nucleocytoplasmic proteins is catalyzed by *O*-linked GlcNAc transferase (OGT) [1]. A number of proteins residing in cytoplasm and nucleus have been known to be modified by *O*-GlcNAc, which results in either direct or indirect effects on biological processes in mammalian cells [2–4]. Null mutation of OGT gene has revealed that protein *O*-GlcNAcylation is essential for cell viability [5]. Recently, protein *O*-GlcNAcylation has been reported to involve in cellular sensing of nutrients [6], toxic and heat stress [7,8]. Until now, many cases that protein *O*-GlcNAcylation interplays with phosphorylation have been reported, suggesting that *O*-GlcNAc can be immersed as a regulatory factor in cellular signaling cascades [9].

OGT is comprised of TPR domain at N-terminal half and CAT domain at C-terminal half [10]. cDNAs encoding OGT have been cloned in mammals, such as human, rat, and mouse [11], and identified in other species, such as *Caenorhabditis elegans*, *Drosophila melanogastor*, *Arabidopsis thaliana*, and zebrafish [12]. Moreover, three isoforms of OGT, such as ncOGT, mOGT, and sOGT, were identified in mammalian species [10] and six different variants of OGT were identified during zebrafish development [12]. Interestingly, it has been reported that mOGT is targeted to mitochondria while ncOGT and sOGT are localized in nucleocytoplasm [13,14].

These OGT isoforms contain the identical CAT domain at C-terminus, respectively, but differ in the number of TPR motifs at N-terminus [15]. Single TPR consists of 34 amino acid residues by which a helical structure can be formed [16]. TPR domain contains multiple repeats of TPR superhelix and is believed to be involved in substrate binding specifically [17]. Crystal structure of TPR domain has revealed Asn-ladder superhelix as in case of importin-α, demonstrating that TPR domain of OGT seems to recognize some spacious conformation of target proteins [18]. Recently, it has been shown that OGT isoforms may recognize target proteins in unique fashions in vitro [15]. At present, whether the functional role of OGT isoforms is unique or redundant in cells is not understood and also, substrate specificity of each isoform is still ambiguous in vivo.

In this report, to investigate the capability of target recognition of OGT, co-expression system of mammalian OGT with its target substrates was established in *Escherichia coli*. Because OGT activity is lacking in prokaryotic cells [19], it is obvious that all endogenous proteins in *E. coli* are deficient of *O*-GlcNAcylation. Here, we found that sOGT was self-*O*-GlcNAcylated. In addition, we provide evidence that Sp1 modulates target recognition of ncOGT by which mammalian *O*-GlcNAcylation is functionally ongoing to endogenous bacterial proteins.

Abbreviations: OGT, *O*-GlcNAc transferase; ncOGT, nucleocytoplasmic *O*-GlcNAc transferase; mOGT, mitochondria *O*-GlcNAc transferase; sOGT, short *O*-GlcNAc transferase; TPR, tetratrico repeat domain.

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Materials and methods

DNA construction and protein expression. Total RNA was purified from PC12, A431, and H293 cells using RNAzol B (Tel-Test, Texas, USA) according to manufacturer's protocols and single stranded cDNA libraries were synthesized using random hexamers (Takara) by 200 u of M-MLV reverse transcriptase (Promega) in 50 mM Tris–HCl (pH 8.3) containing 75 mM KCl, 3 mM $MgCl_2$, 0.5 mM dNTPs, and RNase inhibitor (Takara) for 1 h at 37 °C according to manufacturer's instructions. cDNA encoding OGT isoforms (ncOGT, mOGT, and sOGT), TPR domain, and CAT domain was synthesized by PCR (Dice PCR thermal cycler, Takara) using 50 pmol of gene-specific primers, 0.2 mM dNTPs, and 1.25 u of exTaq polymerase (Takara). cDNAs of OGT enzymes were cloned in pMAL-c2 expression vector (New England Biolabs), respectively. cDNAs of target proteins including p62, Sp1, p53, and $EGFR^{cyto}$ were also synthesized by PCR using gene-specific primers and ExTaq polymerase and were cloned in pET28a(+) expression vector (Novagen). For establishment of coexpression system of OGT and target substrates in vivo, cDNAs cloned in pMAL-c2 and in pET28a(+) were either separately transformed or co-transformed into BL21 (DE3) codon plus competent cells (Stratagene) and single clones were selected under 100 μg/ml of ampicillin (pMAL-c2 system) or, and 50 μg/ml of kanamycin (pET28a(+) system).

SDS–PAGE and immunoblotting. All selected clones were cultured in LB liquid media containing antibiotics (kanamycin and ampicillin) without or with 0.5 mM IPTG induction for 3 h. Cells were harvested, cell lysates were subjected to 10% SDS–PAGE, and analyzed by immunoblotting as described previously [8]. Briefly, immunoblotting was performed on electro-transferred nitrocellulose membrane using His-tag (Santa Cruz), T7-tag (Novagen), MBP (Santa Cruz), Sp1 (Santa Cruz), and RL-2 (Alexis, Switzerland) primary antibody (1:10,000), respectively, in TBST buffer (10 mM Tris (pH 7.6) containing 150 mM NaCl and 0.05% Tween 20) followed by binding of HRP, conjugated secondary antibody and immuno-signals of reaction product were developed on Super RX film (Fuji) using SuperSignal West Pico chemiluminescent substrates (Pierce).

Gene-specific primer sequences. Standard PCR amplifications were carried out using the following oligonucleotides. Restriction enzyme site was underlined, and S and AS designate sense and anti-sense primers, respectively.

1. ncOGT (full length of rat nucleocytoplasmic OGT), S/AS: 5′-AAAGGATCCATGGCGTCTTCCGTGGGCAAC-3′/5′-TTTGTCGACTCAGGCTGACTCAGTGACTTC-3′
2. mOGT (full length of rat mitochondrial OGT) S:5′-AAAGGATCCATGCTGCAGGGTCACTTTTGG-3′
3. sOGT (full length of rat short OGT) S: 5′-CACGGATCCATGCACTATAAGGAAGCC-3′
4. TPR (N-terminal half for tetratrico repeat domain of ncOGT) AS: 5′-TGGGTCGACTCAACAGACAATCTGTAGGC -3′
5. CAT (C-terminal half for catalytic domain of ncOGT), S: 5′-CTCGGATCCGATTGGACAGACTATG -3′
6. p62 (full length of rat nucleopore protein), S/AS: 5′-CCGGATCCATGAGTGGGTTTAACTTTGG-3′/5′-CCCAAGCTTCTAGTCAAAGGCAATGCGCAG-3′
7. p53 (full length of human tumor suppressor), S/AS: 5′-TTTGGATCCATGGAGGAGCCGCAGTCAG-3′/5′-TTTGTCGACTCAGTCTGAGTCAGGCCC-3′
8. Sp1 (full length of human transcriptional factor), S/AS: 5′-ACCCAGATCTATGAGCGACCAAGATCAC-3′/5′-AAACTCGAGTCAGAAGCCATTGCCACTG-3′
9. EGFRcyto (cytosolic domain of human EGF receptor), S/AS: 5′-ATAAGATCTATGCGAAGGCGCCACATCG-3′/5′-CGCGCTCGAGTCATGCTCCAATAAATTCACTGC-3′.

OGT assay and β-N-acetylglucosaminidase treatment. OGT (ncOGT, mOGT, and sOGT) activities were assayed in vitro using target proteins and either cold UDP-GlcNAc or radiolabeled UDP-[^{3}H] GlcNAc (Perkin-Elmer Life Science) in buffer as described previously [8]. Radioactive-signals and immuno-signals were developed directly on Super RX film (Fuji) or using SuperSignal West Pico chemiluminescent substrates (Pierce) as described above. *O*-GlcNAc residues transferred by OGT reaction were cleaved by β-hexoseaminidase treatment. Samples were treated with 10–40 mU of β-GlcNAc-specific hexoseaminidase (Calbiochem, β1-2,3,4,6-*N*-acetylglucosaminidase) for time-course of several hours to overnight at 37 °C in 50 mM sodium phosphate buffer (pH 5.0).

Immunoprecipitation and pull-down assay. Bacterial clones were frozen and thawed in lysis buffer (20 mM Tris–Cl, pH 7.4, containing 200 mM NaCl, 10 mM β-mercaptoethanol, 1 mM EDTA, and protease inhibitor cocktails (Merck)). The thawed cell lysates were added by lysozyme (1 mg/ml) and PMSF (1 mM), incubated on ice for 30 min, and finally sonicated in ice. Clean supernatants containing soluble proteins were collected after microcentrifugation at 13,000*g* for 20 min, pre-cleaned with Protein A–Sepharose (Pierce), and immunoprecipitated with RL2 or Sp1 antibody (1 μg each) and Protein A-Sepharose for several hours in cold chamber. For pull-down of OGT isoforms, Amylose resin (New England Biolabs) was added to clean supernatants (20 mg of total proteins) and incubated for several hours in cold chamber. Amylose resins were washed several times with lysis buffer and beads were collected by microcentrifugation at 3000 rpm for 1 min.

Thermal resistance analysis. Bacterial clones including mock cells, transformants with enzyme sources in pMAL-c2 vectors or with substrate sources in pET28a(+) vectors, and co-transformants with both sources were grown in LB containing 100 μg/ml ampicillin or/and 50 μg/ml kanamycin. For analysis of thermal resistance, cells in 0.2 ml of LB broth (OD_{600} = 0.5) were heated with 52 °C for 3 h in a shaker bath and after heating, cells were plated on M9 minimal and LB agar containing antibiotics. Viable cells were visually inspected after incubation at 37 °C overnight [20]. As a control, identical bacterial clones in the same condition (0.2ml LB, OD_{600} = 0.5) were not heat treated and plated on agar with serial dilution for visual cell counting. The relative efficiency for cell survival is defined as the ratio of colony number after heating divided by colony number before heating. Cell survival ratio was calculated from the average value of experimental triplicates.

Results

Expression of OGT isoforms in vivo

Recombinant OGT isoforms of ncOGT, mOGT, and sOGT were expressed as a MBP fusion protein using pMAL-c2 vector in BL21(DE3) cells and if necessary, protein expressions were visualized by Coomassie or Ponceau staining before and after IPTG induction, and Western blotting using anti-MBP-antibody. To examine the occurrence of endogenous protein *O*-GlcNAcylation, cell lysates from OGT clones were analyzed by RL2 immunoblotting. Although OGT isoforms were normally expressed as 170, 160, and 120 kDa (Fig. 1C), respectively, neither OGT isoforms nor endogenous bacterial proteins were significantly *O*-GlcNAcylated except that unidentified 120 kDa protein band was strongly *O*-GlcNAcylated only in sOGT-expressing clones (Fig. 1A). Ponceau staining showed that loading cell lysates was constant (Fig. 1B).

To identify whether unidentified *O*-GlcNAcylated 120 kDa protein in sOGT-expressing clones is originated from bacterial proteins, OGT isoforms were re-expressed in pET28a(+) vector system instead of pMAL-c2 vector. OGT isoforms in pET28a(+) vector were expressed as the expected molecular mass of 120, 110,

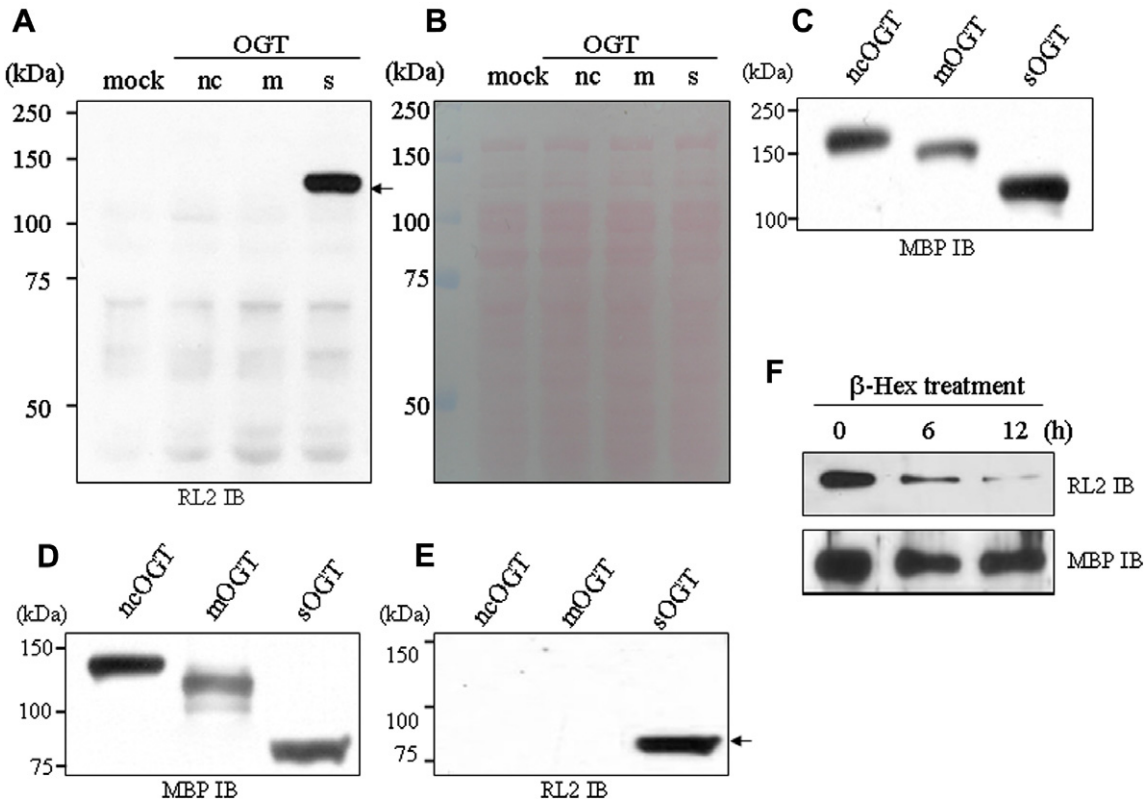

Fig. 1. Auto-*O*-GlcNAcylation of sOGT. Cell lysates from OGT clones expressing three OGT isoforms in pMAL-c2 vector were separated on 10% SDS–PAGE and immunoblotted with *O*-GlcNAc antibody (RL2) (A), stained by Ponceau (B), and immunoblotted with MBP-specific antibody (C). Cell lysates from OGT clones expressing three OGT isoforms in pET28a(+) vector were immunoblotted with MBP-specific antibody (D), and with RL2 (E). Positively blotted protein band by RL2 in pMAL-c2 vector and pET28a(+) vector is indicated by arrow, respectively.

and 80 kDa, respectively (Fig. 1D). Indeed, immunoblot analysis by RL2 showed that *O*-GlcNAcylation was detected on 80 kDa protein in pET28a(+) vector system (Fig. 1E). These results show that this *O*-GlcNAcylated protein appears to be sOGT itself, not bacterial proteins. This self-*O*-GlcNAcylation of sOGT was further confirmed by treatment with GlcNAc-specific β-hexoseaminidase (Fig. 1F).

Coexpression of ncOGT with target substrates in vivo

To examine the capability of target recognition by ncOGT in vivo, we established coexpression system of ncOGT with its substrates in *E. coli*. In this system, enzyme sources (ncOGT, TPR domain (N-terminus half containing 12.5 TPR), and CAT domain (C-terminal half containing catalytic domain) were produced as MBP-fusion protein in pMAL-c2 vector (amp^r) while substrate sources (p62, Sp1, p53 (a positive control), and $EGFR^{cyto}$ (negative control), respectively) were produced in pET28a(+) (kan^r) vector. Expression of recombinant proteins was identified to be an expected molecular mass by Coomassie staining of SDS–PAGE gel and *O*-GlcNAcylation of target substrates was detected by Western blot analysis probing with RL2 antibody (Fig. 2B and D). Protein expression was also checked by immunoblotting using MBP-, His-, and T7-specific antibody (data not shown). p62 and Sp1 were shown to be strongly *O*-GlcNAcylated by ncOGT but not by TPR and CAT (Fig. 2A and C, lane 2) whereas p53 and $EGFR^{cyto}$ showed no *O*-GlcNAcylation by ncOGT, TPR, and CAT (Fig. 2A and C). These results demonstrate that ncOGT are catalytically active in *E. coli* and also, bacterial cytosolic environments can supply UDP-GlcNAc enough to proceed mammalian *O*-GlcNAcylation. Nevertheless, mammalian OGT seems to not recognize most, not all, bacterial cytosolic proteins as right target substrates. It should be noted that p53 was not able to be substantially *O*-GlcNAcylated by ncOGT under our coexpression system.

Notably, we found that coexpression of ncOGT with Sp1 produced multiple *O*-GlcNAcylation patterns on many protein bands including Sp1 band and these patterns include large and small molecular weight proteins compared to Sp1 (Fig. 2C, lane 2). This pattern was not observed in coexpression of p62 and ncOGT (Fig. 1A, lane 2). These results strongly suggest that OGT may initiate *O*-GlcNAcylation toward endogenous bacterial proteins in the presence of Sp1.

Coexpression of OGT isoforms with target substrates in vivo

To further investigate Sp1 modulation of OGT action, we coexpressed three OGT isoforms with Sp1 and other targets such as p62, p53, and $EGFR^{cyto}$ in *E. coli*. Coexpressed p62 before and after IPTG induction was shown to be differentially *O*-GlcNAcylated depending on OGT isoforms, demonstrating that p62 was most strongly *O*-GlcNAcylated by ncOGT (Fig. 3A and C). Coexpressed Sp1 before and after IPTG induction was shown to be similarly *O*-GlcNAcylated as in case of coexpressed p62 (Fig. 3D and F). Expression of OGT isoforms and target proteins was confirmed by Ponceau staining after IPTG induction (Fig. 3B and E). p53 and $EGFR^{cyto}$ were not *O*-GlcNAcylated by any OGT isoforms (Fig. 3B and F). Moreover, self-*O*-GlcNAcylation of sOGT was not shown under IPTG induction (Fig. 3B and E, C, and F). Regardless of IPTG induction, coexpression of Sp1 with ncOGT gave rise to multiple *O*-GlcNAcylation patterns (Fig. 3D and F), but not with mOGT and sOGT. Therefore, it is very likely that Sp1 may specifically modulate ncOGT action.

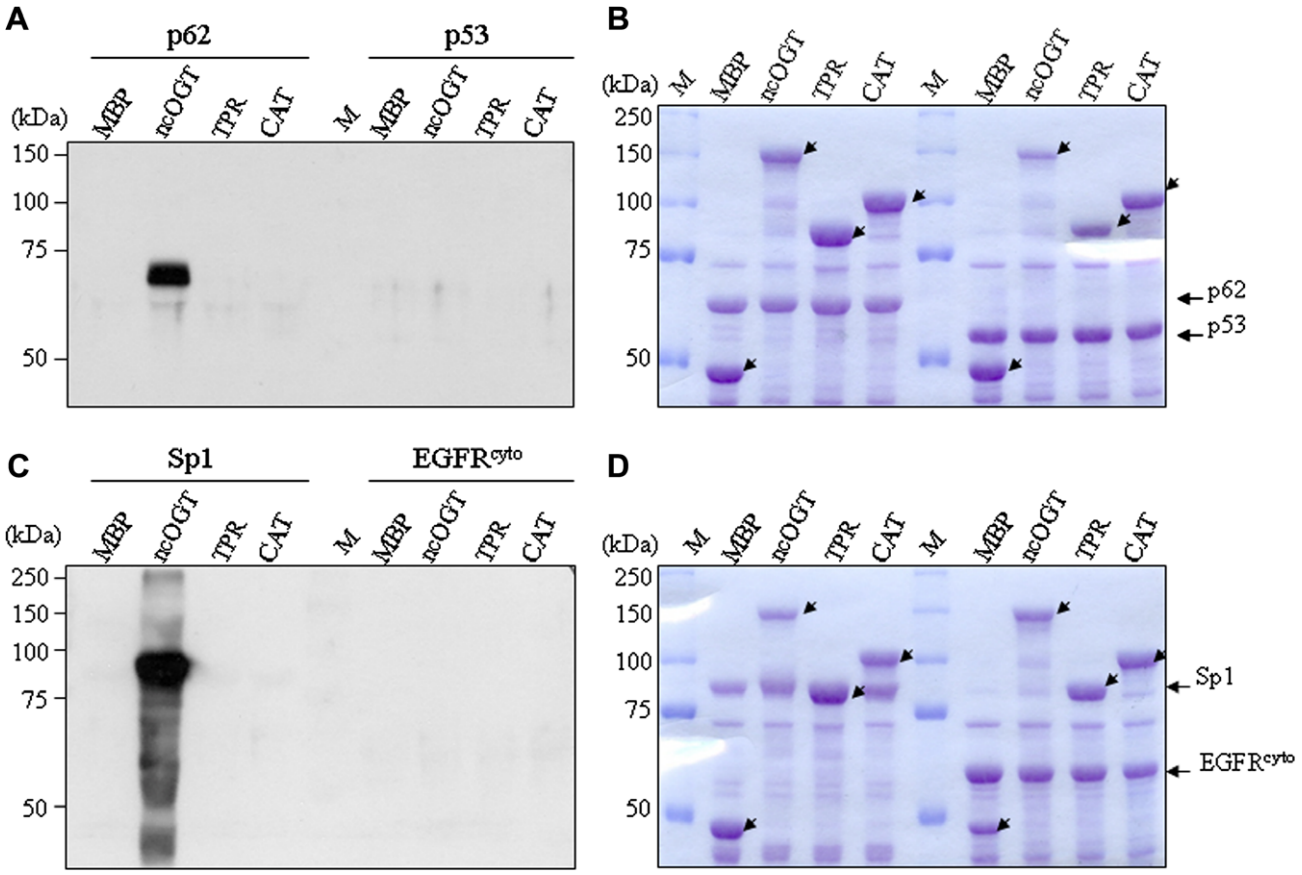

Fig. 2. Coexpression of OGT with targets in vivo. OGT enzyme sources (ncOGT, TPR, and CAT) were coexpressed in pMAL-c2 vector with substrates (p62, p53, and Sp1 as a positive and $EGFR^{cyto}$ as a negative control) in pET28a(+) vector. Whole cell lysates from coexpressing clones were immunoblotted with RL2 (A, C) and stained with Coomassie (B, D). Maltose binding protein (MBP, pMAL-c2 vector only) and protein bands corresponding to OGT enzyme sources and substrates are indicated by tilted and horizontal arrows, respectively.

Sp1-mediated modulation of ncOGT activity in vitro and Co-interaction of Sp1 with ncOGT

To determine the identity of multiple *O*-GlcNAcylation patterns in more detail, we performed in vitro approaches as follows. Ni–agarose affinity purified Sp1 from Sp1-expressing clone (in pET28a(+) vector system) was incubated with cell lysates from ncOGT-expressing clone (in pMAL-c2 vector system) for time-course reaction in the presence of UDP-GlcNAc, demonstrating that purified Sp1 can also modulate ncOGT action in vitro while no significant degradation of Sp1 was observed during the time-course incubation (Fig. 4A and B). To explore the co-interaction of Sp1 with OGT isoforms, immunoprecipitation and pull-down assay were performed reciprocally. Three OGT isoforms were pulled-down by amylose–resins and Sp1 was immunoprecipitated from cell lysates of coexpressing clones, Sp1/ncOGT, Sp1/mOGT, and Sp1/sOGT, respectively (Fig. 4C and D). Sp1 was co-precipitated only with ncOGT, not with mOGT and sOGT (Fig. 4C, upper panel) and reciprocally, only ncOGT was co-precipitated with Sp1 (Fig. 4D, upper panel). These results indicate that Sp1 co-interacts with ncOGT with high affinity in vivo.

Thermal resistance of the O-glyco-bacteria

Now, the bacterial clone that coexpresses Sp1 and ncOGT is designated as the *O*-glyco-bacteria, in which mammalian *O*-GlcNAcylation is endogenously ongoing to procaryotic proteins. We investigated whether the *O*-glyco-bacteria possess physiological stability against hyperthermal stress, demonstrating that thermal resistance at a temperature as high as 52 °C was strongly observed in the *O*-glyco-bacteria compared to other bacterial clones (Fig. 4E). Weak thermal resistance was observed in Sp1/mOGT-coexpressing clones, indicating that Sp1 may interact with mOGT with low affinity in vivo. Together, these results indicate that *O*-GlcNAcylation on endogenous proteins in the *O*-glyco-bacteria can attenuate cytotoxicity by which enhanced thermal resistance is generated.

Discussion

cDNAs encoding three OGT isoforms in mammalian cells could be generated by alternative splicing and their translated products have been detected in HeLa cells [10]. Also, it has shown that six OGT variants in zebrafish are produced by alternative splicing and developmentally regulated [12]. These studies suggest that OGT isoforms or OGT variants might be specifically involved in cellular functions beyond the species. In particular, ncOGT and sOGT appear to function in nucleocytoplasm while mOGT is solely localized in mitochondria [14]. Until now, nearly hundreds of nucleocytoplasmic proteins have been known to be *O*-GlcNAcylated [21], however, little is understood about target recognition by OGT isoforms in vivo.

Neo-expressed ncOGT in *E. coli* was confirmed to be catalytically active when it was co-expressed with p62, demonstrating that cytosolic environment of *E. coli* could supply UDP-GlcNAc enough to exert ncOGT action of protein *O*-GlcNAcylation. Coexpres-

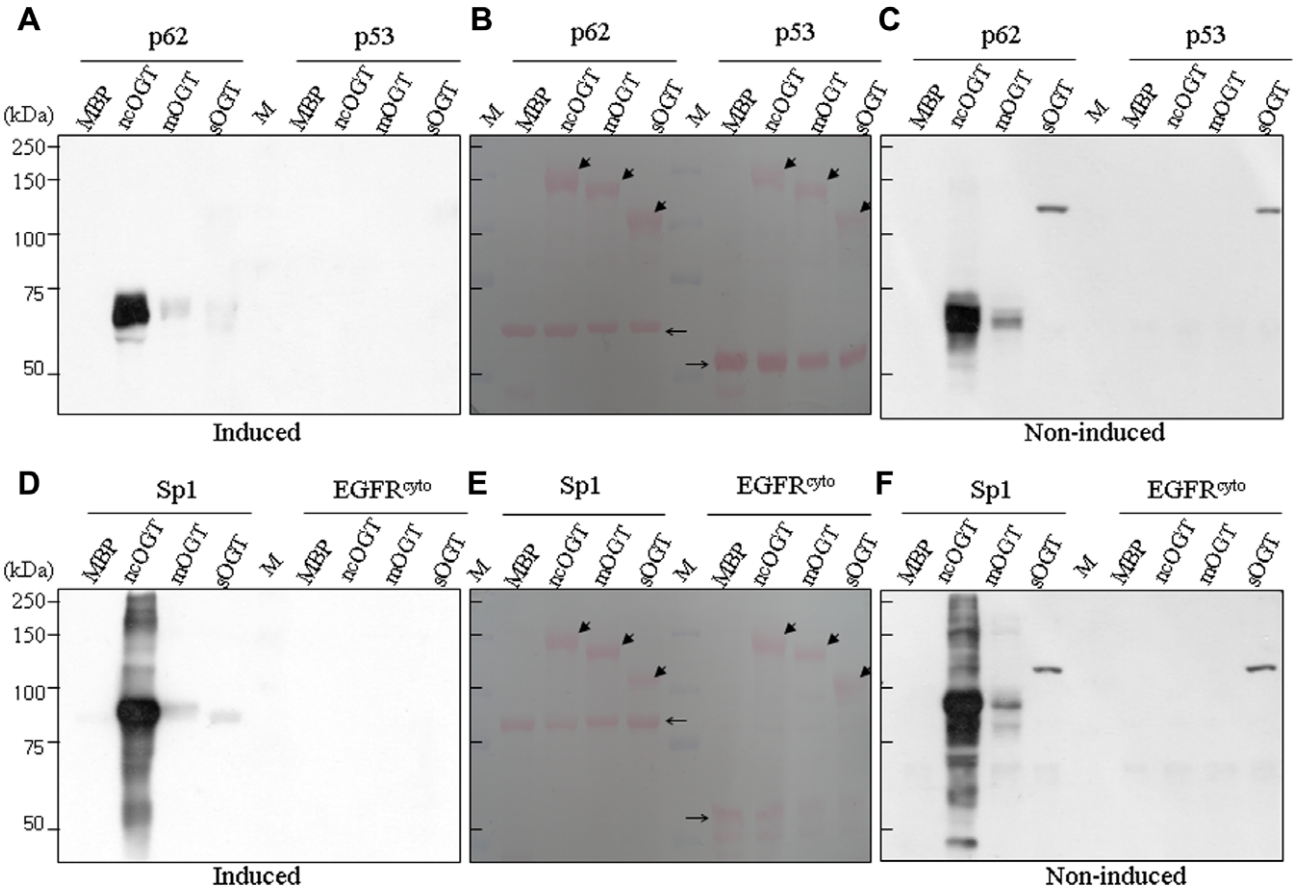

Fig. 3. Coexpression of three OGT isoforms with targets in vivo. Three OGT isoforms were coexpressed in pMAL-c2 vector with substrates in pET28a(+) vector. Cell lysates from IPTG-induced (A, D) and -non-induced (C, F) clones were immunoblotted with RL2. Cell lysates from IPTG induced clones were stained with Ponceau (B, E) and protein bands corresponding to OGT isoforms and substrates are indicated by tilted and horizontal arrows, respectively.

sed p62 with ncOGT, mOGT, and sOGT was differentially *O*-GlcNAcylated depending on OGT isoforms and this observation in vivo is consistent with previous study in vitro [19]. However, catalytic action of OGT isoforms toward endogenous bacterial proteins appears not to occur since no bacterial proteins were not significantly *O*-GlcNAcylated under these conditions. Unexpectedly, we found that 120 kDa protein was strongly *O*-GlcNAcylated in pMAL-c2/sOGT-transformed cells, but neither in pMAL-c2/ncOGT- nor pMAL-c2/mOGT-transformed cells. To investigate this *O*-GlcNAcylation, OGT isoforms were re-expressed in pET28a(+) expression vector. Indeed, 120 kDa protein *O*-GlcNAcylated in pMAL-c2/sOGT system was shifted down to 80 kDa protein in pET28a(+)/sOGT system. In fact, pMAL-c2/sOGT-transformed cells produce sOGT as a MBP-tagged fusion protein corresponding to molecular mass of 120 kDa whereas pET28a(+)/sOGT-transformed cells produce sOGT as 80 kDa protein. These data strongly suggest that sOGT itself is *O*-GlcNAcylated since it is very unlikely that molecular mass of bacterial protein that is *O*-GlcNAcylated by sOGT will be changed from 120 kDa to 80 kDa depending on sOGT expression in different vector system. Therefore, the *O*-GlcNAcylated 120 kDa protein in pMAL-c2/sOGT- and 80 kDa protein in pET28a(+)/sOGT-transformed cells were not originated from endogenous bacterial proteins. This result seems to be contradictory to that of other previous study that sOGT has been shown not to be auto-*O*-GlcNAcylated [19].

Coexpression of OGT isoforms with Sp1 resulted in similar patterns of differential *O*-GlcNAcylation of Sp1 depending on OGT isoforms as in case of p62 coexpression. But, it was of surprise to note that coexpression of ncOGT with Sp1 gave rise to significant *O*-GlcNAcylation of endogenous bacterial proteins. No such observation occurs in any case of the coexpression of target proteins, such as p62 and p53, indicating that Sp1 may specifically modulate the ncOGT to newly recognize target substrates. Protein interactions between OGT isoforms and Sp1 were further investigated by reciprocal co-immunoprecipitation, demonstrating that ncOGT was found to strongly bind with Sp1 but mOGT and sOGT were not. Sp1 modulation of ncOGT action was further confirmed by in vitro reaction containing purified Sp1 and UDP-GlcNAc, demonstrating that *O*-GlcNAcylation of bacterial proteins was significantly increased up to 6 h incubation whereas no degradation of Sp1 was observed. Considering that target recognition of ncOGT is exerted by TPR domain, Sp1 binding to ncOGT may generate likely conformational changes in TPR domain by which new proteins are recognized as right targets to be *O*-GlcNAcylated.

By coexpression of ncOGT and Sp1 in *E. coli*, we, for the first time, generate the *O*-glyco-bacteria in which mammalian *O*-GlcNAcylation is functionally ongoing to endogenous procaryotic proteins. Although the molecular mechanism of Sp1 modulation of ncOGT activity needs further study, we strongly feel that there must be some specific changes in cellular physiology of the *O*-glyco-bacteria. Interestingly, we found that the *O*-glyco-bacteria showed enhanced thermal resistance, demonstrating that endogenous protein *O*-GlcNAcylation allows cells to survive under hyperthermal stress at a temperature as high as 52 °C. We have previously demonstrated that OGT mediated protein *O*-GlcNAcylation functions as a chaperone-like role during heat stress in mammalian system [8]. It is clear that defense role of protein *O*-GlcNAcylation against heat stress may be reproduced in the *O*-glyco-bacteria as in mammalian system. The

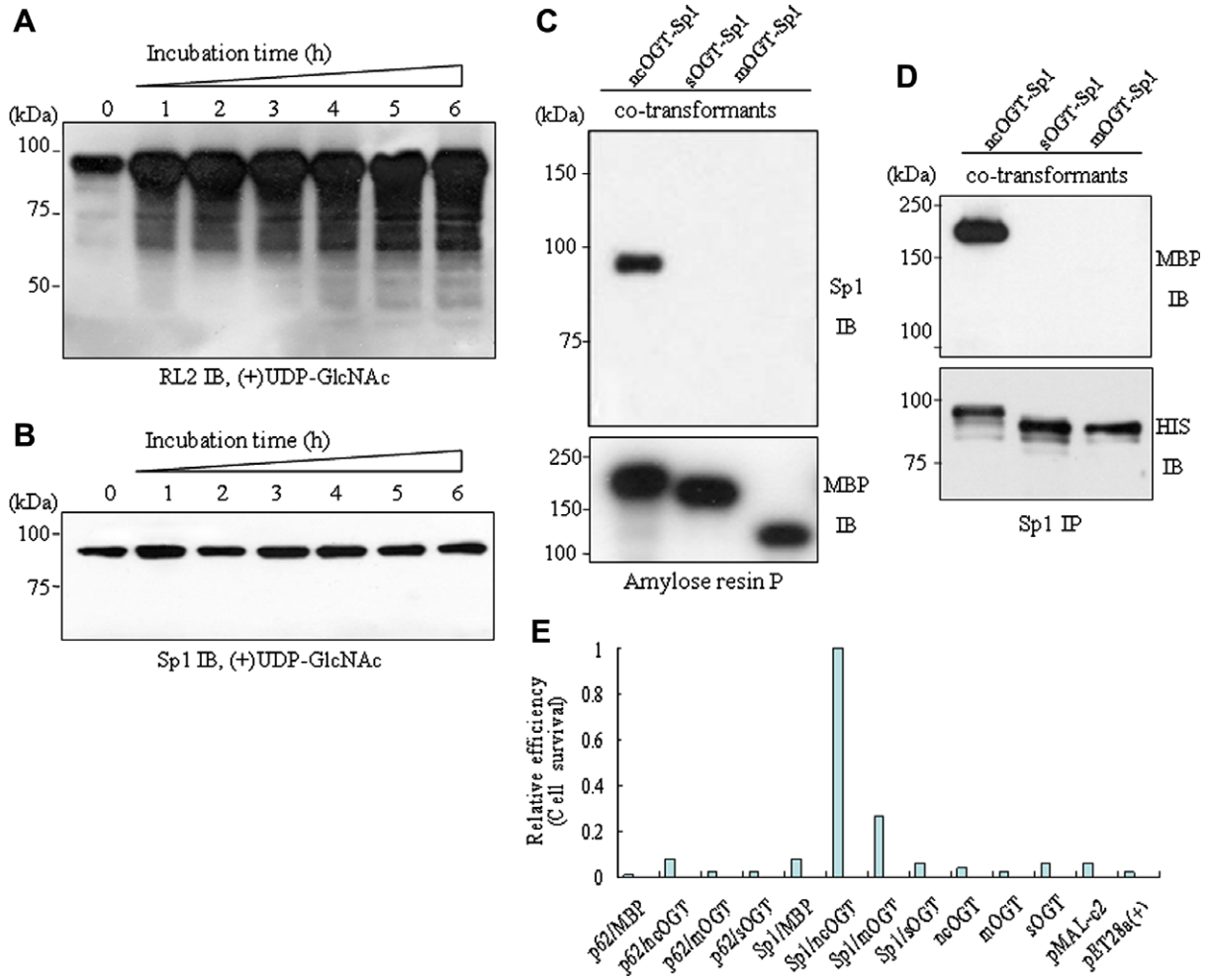

Fig. 4. Modulation of ncOGT activity by Sp1 in vitro and interaction of Sp1 with ncOGT generates thermal resistance. Sp1 was expressed in pET28a(+) vector and ncOGT was expressed pMAL-c2 vector. Sp1 was affinity purified by Ni-agarose from cell lysates of Sp1-expressing clone. Purified Sp1 was mixed with cell lysates from ncOGT-expressing clone and incubated in time-course for *O*-GlcNAcylation reaction in the presence of UDP-GlcNAc (+) followed by immunoblotting with RL2 (A) and Sp1-specific antibody (B). Three OGT isoforms were pulled down by amylose–resin (C) or immunoprecipitated by Sp1 antibody (D) from cell lysates of coexpressing clones of three OGT isoforms and Sp1 followed immunoblotting with Sp or MBP antibody (A, upper and lower panel), and MBP or His antibody (B, upper and lower panel). (E) Thermal resistance at 52 °C was examined in Sp1-ncOGT-coexpressing clone and compared to that in other bacterial clones. Cell survival ratio is the average value of triplicates.

identity of *O*-GlcNAcylated proteins in the *O*-glyco-bacteria remains to be determined. The present study will give insight into a novel regulatory role of Sp1 for controlling OGT action to alter cellular states of protein *O*-GlcNAcylation.

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References

[1] G.W. Hart, *O*-linked glycosylation of nuclear and cytoskeletal proteins, Annu. Rev. Biochem. 66 (1997) 315–335.
[2] K. Vosseller, L. Wells, G.W. Hart, Nucleocytoplasmic *O*-glycosylation: *O*-GlcNAc and functional proteomics, Biochimie 83 (2001) 575–581.
[3] L. Well, K. Vosseller, G.W. Hart, Glycosylation of nucleocytoplasmic proteins: signal transduction and *O*-GlcNAc, Science 291 (2001) 2376–2378.
[4] X. Yang, F. Zhang, J.E. Kudlow, Recruitment of *O*-GlcNAc transferase to promoters by corepressor mSin3A: coupling protein *O*-GlcNAcylation to transcriptional repression, Cell 110 (2002) 69–80.
[5] R. Shafi, S.P. Iyer, L.G. Ellis, N. O'Donnell, K.W. Marek, G.W. Chui, G.W. Hart, The *O*-GlcNAc transferase gene resides in the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny, Proc. Natl. Acad. Sci. USA 97 (2000) 5735–5739.
[6] N.E. Zachara, G.W. Hart, *O*-GlcNAc a sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress, Biochim. Biophys. Acta 1673 (2004) 13–28.
[7] N.E. Zachara, N. O'Donnell, W.D. Cheung, J.J. Mercer, J.D. Marth, G.W. Hart, *O*-GlcNAc a sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress. A survival response of mammalian cells, J. Biol. Chem. 279 (2004) 30133–30142.
[8] K.C. Sohn, K.-Y. Lee, J.E. Park, S.-I. Do, OGT functions as a catalytic chaperone under heat stress response: a unique defense role of OGT in hyperthermia, Biochem. Biophys. Res. Commun. 22 (2004) 1045–1051.
[9] K. Vosseller, K. Sakabe, G.W. Hart, Diverse regulation of protein function by *O*-GlcNAc: a nuclear and cytoplasmic carbohydrate post-translational modification, Curr. Opin. Chem. Biol. 6 (2002) 851–857.
[10] J.A. Hanover, S. Yu, W.B. Lubas, S.H. Shin, M. Ragano-Caracciola, J. Kochran, D.C. Love, Mitochondrial and nucleocytoplasmic isoforms of *O*-linked GlcNAc transferase encoded by a single mammalian gene, Arch. Biochem. Biophys. 409 (2003) 287–297.
[11] W.A. Lubas, D.W. Frank, M. Krause, J.A. Hanover, GlcNAc transferase is a conserved nucleocytoplasmic protein containing tertratricopeptide repeats, J. Biol. Chem. 272 (1997) 9316–9324.
[12] K.C. Sohn, S.-I. Do, Transcriptional regulation and *O*-GlcNAcylation activity of zebrafish OGT during embryogenesis, Biochem. Biophys. Res. Commun. 337 (2005) 256–263.
[13] Y. Akimoto, L.K. Kreppel, H. Hirano, G.W. Hart, Localization of the *O*-linked *N*-acetylglucosamine transferase in rat pancreas, Diabetes 48 (1999) 2407–2413.

[14] D.C. Love, J. Kochran, R.L. Cathey, S.-H. Shin, J.A. Hanover, Mitochondrial and nucleocytoplasmic targeting of *O*-linked GlcNAc transferase, J. Cell Sci. 116 (2002) 647–654.
[15] B.D. Lazarus, D.C. Love, J.A. Hanover, Recombinant *O*-GlcNActransferase isoforms: identification of *O*-GlcNAcase YES tyrosine kinase and Tau as isoform-specific substrates, Glycobiology 16 (2006) 415–421.
[16] F.H. Liu, S.J. Wu, S.M. Hu, C.D. Hsiao, C. Wang, Specific interactin of the 70-kDa heat shock cognate protein with the tetratricopeptide repeats, J. Biol. Chem. 274 (1999) 34425–34432.
[17] S.P. Iyer, G.W. Hart, Roles of the tetratricopeptide repeat domain in *O*-GlcNAc transferase targeting and protein substrate specificity, J. Biol. Chem. 278 (2003) 24608–24616.
[18] M. Jinek, J. Rehwinkel, D. Lazarus, E. Izaurralde, J.A. Hanover, E. Conti, The superhelical TPR-repeat domain of *O*-linked GlcNAc transferase exhibits structural similarities to importin α, Nat. Struct. Mol. Biol. 11 (2004) 1001–1007.
[19] W.A. Lubas, M. Smith, C.M. Starr, J.A. Hanover, Analysis of nuclear pore protein p62 glycosylation, Biochemistry 34 (1995) 1687–1694.
[20] I.-T. Chow, F. Baneyx, Coordinated synthesis of the two ClpB isoforms improves the ability of *Escherichia coli* to survive thermal stress, FEBS Lett. 579 (2005) 4235–4241.
[21] S.A. Whelan, G.W. Hart, Proteomic approaches to analyze the dynamic relationships between nucleocytoplasmic protein glycosylation and phosphorylation, Circ. Res. 93 (2003) 1047–1058.