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# The Essential Escherichia coli Apolipoprotein N -Acyltransferase (Lnt) Exists as an Extracytoplasmic Thioester Acyl-Enzyme Intermediate

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Thioester acyl-Lnt intermediate

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**THE ESSENTIAL *E. COLI* APOLIPOPROTEIN N-ACYLTRANSFERASE (LNT) EXISTS AS  
AN EXTRACYTOPLASMIC THIOESTER ACYL-ENZYME INTERMEDIATE<sup>‡</sup>**

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Running title: Thioester acyl-Lnt intermediate

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Thioester acyl-Lnt intermediate

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24 **FOOTNOTES**

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28

29 Abbreviations used in the text: methoxypoly(ethylene glycol)-maleimide (malPEG), hydroxyl amine  
30 (HA).

31

32

33 **ABSTRACT**

34

35 *Escherichia coli* apolipoprotein N-acyltransferase (Lnt) transfers an acyl group from *sn*-1-  
36 glycerolphospholipid to the free  $\alpha$ -amino group of the N-terminal cysteine of apolipoproteins,  
37 resulting in mature triacylated lipoprotein. Here we report that the Lnt reaction proceeds  
38 through an acyl enzyme intermediate in which a palmitoyl group forms a thioester bond with the  
39 thiol of active site residue C387 that was cleaved by neutral hydroxylamine. Lnt(C387S) also  
40 formed a fatty acyl intermediate that was resistant to neutral hydroxylamine treatment,  
41 consistent with formation of an oxygen-ester linkage. Lnt(C387A) did not form an acyl enzyme  
42 intermediate and, like Lnt(C387S), did not have any detectable Lnt activity, indicating that  
43 acylation can not occur at other positions in the catalytic domain. The existence of this thioacyl-  
44 enzyme intermediate allowed us to determine whether essential residues in the catalytic domain  
45 of Lnt affect the first step of the reaction, the formation of the acyl enzyme intermediate, or the  
46 second step in which the acyl chain is transferred to apolipoprotein substrate. In the catalytic  
47 triad, E267 is required for the formation of the acyl-enzyme intermediate, indicating its role in  
48 enhancing the nucleophilicity of C387. E343 is also involved in the first step but is not in close  
49 proximity to the active site. W237, Y388 and E389 play a role in the second step of the reaction  
50 since acyl-Lnt is formed but N-acylation does not occur. The data presented allow discrimination  
51 between the functions of essential Lnt residues in catalytic activity and substrate recognition.

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53

### Thioester acyl-Lnt intermediate

54 Lipoproteins are major components of the bacterial cell envelope; in *E. coli*, Braun's lipoprotein  
55 (Lpp) at  $\sim 10^6$  copies, is easily the most numerous protein. Lipoprotein signal sequences terminate in a  
56 lipobox motif, L(A/V)<sub>-4</sub>-L<sub>-3</sub>-A(S)<sub>-2</sub>-G(A)<sub>-1</sub>-C<sub>+1</sub>, identifying a Cys residue as the site of post-  
57 translational modification processing (Fig. 1a) (1). First, phosphatidylglycerol::apolipoprotein  
58 diacylglyceryl transferase (Lgt) adds a phosphatidylglycerol (PG) -derived *sn*-1,2-diacylglyceryl group  
59 via a thioether bond. Next, the signal sequence is removed by prolipoprotein signal peptidase (LspA, or  
60 signal peptidase II), liberating the  $\alpha$ -amino group of C<sub>+1</sub>. In Gram-negative bacteria and in  
61 *Mycobacteria* (2), a third step, *N*-acylation of diacylglyceride C<sub>+1</sub> by apolipoprotein *N*-acyltransferase  
62 (Lnt), follows, generating the mature tri-acylated lipoprotein. *N*-acylation is required for engaging the  
63 Lol machinery (3), which either transfers the lipoprotein to the inner leaflet of the outer membrane or  
64 leaves it tethered in the outer leaflet of the inner membrane. All three processing enzymes, as well as  
65 the Lol system, are essential. Despite its central role in the generation of the bacterial envelope, the  
66 catalytic mechanism of Lnt is largely unknown. The reaction, in which the carbonyl group of *sn*-1-  
67 glycerolphospholipid is linked to the free  $\alpha$ -amino group of apolipoprotein, involves the formation of  
68 an acyl enzyme intermediate, analogous to reactions described for members of the nitrilase  
69 superfamily, to which Lnt belongs by virtue of the similarity of its largest periplasmic domain (Fig. 1b)  
70 (4). We previously identified the catalytic triad, E267-K335-C387, and residues W237, E343, Y388  
71 and E389 in the periplasmic domain as essential for Lnt activity in *E. coli* (5). Here we describe  
72 genetic, physiological, and biochemical experiments aimed at further defining Lnt catalysis.

73

## 74 **EXPERIMENTAL PROCEDURES**

75

76 *Strains, plasmids and growth media.* Strains PAP105, MC4100 and MG1655 have been described (5,  
77 6). Strains carrying plasmids were grown in LB with 100  $\mu$ g/ml ampicillin.

Thioester acyl-Lnt intermediate

78

79 *Induction of lnt genes.* Strains were grown at 37°C with shaking in LB to OD<sub>600</sub> = 0.2. Expression of  
80 *lnt* from the p<sub>lac</sub> promoter in pUC18 plasmids and from the p<sub>ara</sub> promoter of the pBAD18 constructs was  
81 induced with 1 mM isopropyl-β-thiogalactopyranoside (IPTG), and 0.2% L-arabinose, respectively.  
82 The final OD<sub>600</sub> was measured after 1 h of induction at 37°C, at which time the cultures were placed on  
83 ice.

84

85 *Standard DNA manipulation.* Plasmid pCHAP7530, encoding Lnt with a double c-Myc tag at the C-  
86 terminus, has been described previously (5). Site-directed mutagenesis was performed with pairs of  
87 synthetic oligonucleotides (data not shown) using a two-step polymerase chain reaction based on the  
88 Quick-change site-directed mutagenesis protocol (Stratagene) with pCHAP7530 as template DNA. All  
89 constructs were verified by sequencing. Fragments containing *lnt* in pUC18 derivatives were digested  
90 with EcoRI and HindIII and inserted into pBAD18 for p<sub>ara</sub> controlled expression (7).

91

92 *Hydroxylamine treatment.* Bacteria (1.6 ml) were centrifuged at 16,000 g for 5 min and resuspended in  
93 1 ml Phosphate Buffer (PB) (50 mM KPi pH 7.0). Samples were split into two tubes and centrifuged at  
94 16,000 g for 5 min. One pellet was resuspended in 1M neutral hydroxylamine (HA; made immediately  
95 before use by dissolving 1.4 g NH<sub>2</sub>OH in 20 ml H<sub>2</sub>O and adjusting to pH 7.0 by gradual addition of  
96 32% NaOH) containing 1% SDS. The second pellet was treated identically except 1M Tris-HCl pH 7.0  
97 containing 1% SDS was used instead of HA. After incubation for 30 min at room temperature, proteins  
98 were precipitated by incubation with 10% TCA for 30 min on ice, collected by centrifugation at 16,000  
99 g for 15 min at 4°C, washed twice with acetone, and resuspended either in PEG buffer (see below) for  
100 alkylation with malPEG, or in SDS sample buffer for analysis by gel electrophoresis (see below).

101

Thioester acyl-Lnt intermediate

102 *Alkylation with MalPEG.* Pellets were air-dried and resuspended in 100  $\mu$ l PEG buffer (1M Tris-HCl  
103 pH 7.0, 10 M Urea, 1% SDS and 1mM EDTA). MalPEG was added to all samples to 0.2 mM and they  
104 were then incubated for 30 min at room temperature. Cold 100% ethanol (1.5 ml) was added and  
105 proteins were precipitated at -20°C. Protein samples were centrifuged at 16,000 g for 15 min at 4°C.  
106 Supernatants were carefully removed and pellets were dried at room temperature and then resuspended  
107 in SDS sample buffer and heated at 100°C for 5 min prior to analysis by gel electrophoresis and  
108 immunoblotting.

109

110 *<sup>3</sup>H-palmitate labeling of whole cells and Fluorography.* Overnight cell cultures were diluted 100 fold  
111 in 5 ml fresh medium and grown at 37°C to OD<sub>600</sub> = 0.2. <sup>3</sup>H-palmitate was added to 100  $\mu$ Ci per ml  
112 and *lnt* expression was induced simultaneously with 0.2%-L-arabinose. The cultures were incubated for  
113 a further 60 min at 37°C. Harvested cells were treated with HA as described above. Proteins were  
114 separated on 10% polyacrylamide-SDS gels. Gels were fixed in 25% isopropanol + 10% acetic acid for  
115 30 min, washed twice in H<sub>2</sub>O for 15 min and incubated in Amplify (GE Healthcare) for 20 min. The  
116 dried gels were exposed to Kodak BioMax XAR film at -80°C.

117

118 *SDS-PAGE, immunoblotting.* For the preparation of whole cell extracts, 1 ml of cells were centrifuged  
119 for 5 min at 16,000 g. Cell pellets were resuspended in SDS sample buffer containing 4 mM DTT and  
120 heated for 5 min at 100°C. Protein samples were loaded at an equivalent of 0.1 OD<sub>600</sub> units and  
121 separated in 10% polyacrylamide SDS-PAGE gels. After transfer onto nitrocellulose membranes,  
122 proteins were detected by incubating the membrane with antibodies against c-Myc (Sigma) followed by  
123 horseradish peroxidase conjugated secondary antibodies to rabbit immunoglobulin G (Amersham).  
124 Secondary antibodies were detected by chemiluminescence (Thermo Scientific) (8).

125

Thioester acyl-Lnt intermediate

126 **RESULTS**

127

128 *The periplasmic thiol of Lnt is blocked in vivo.*

129 The proposed mechanism of *N*-acylation of lipoproteins involves a nucleophilic attack by the  
130 activated thiol of Lnt on the *sn*-1-glycerolphospholipid carbonyl group to generate a lysophospholipid  
131 byproduct and an acyl enzyme intermediate, which is then resolved by the apolipoprotein  $\alpha$ -amino  
132 group. Besides the catalytic cysteine, C387, in the large periplasmic domain, there are two other Cys  
133 residues, neither conserved: C23 in the first transmembrane segment (TMS1) and C62 in TMS3 (Fig.  
134 1b). To facilitate biochemical analysis of Lnt enzymatic function we altered the non-conserved Cys  
135 residues to Ala, both individually and in combination, in the context of an *lnt* allele also encoding a  
136 double c-Myc tag at the cytoplasmic C-terminus. As expected, both C23A and C62A alleles and the  
137 double substitution complemented an *E. coli lnt* conditional null mutant. Surprisingly, when the high-  
138 molecular-weight alkylating agent, methoxypoly(ethylene glycol)-maleimide (malPEG; 5 kDa) was  
139 used to assess the state of the thiols in denatured protein samples prepared from whole cells, both of the  
140 single substitutions exhibited only a single modifiable thiol, and the double substitution had none (Fig.  
141 2). This conclusion was confirmed by showing that modifying the Cys387 to Ser in the parental or  
142 either single substitution had no effect on the number of malPEG-reactive thiols, although as expected  
143 the ability to complement the *lnt* defect was lost. These results indicated that the essential C387 residue  
144 exists in covalently blocked state *in vivo*.

145

146 *Lnt exists as a periplasmic thioester acyl enzyme intermediate.*

147 The most likely covalent modification of Lnt would be an acyl-enzyme intermediate. To test this  
148 idea, we examined the thiol state of Lnt after treatment with neutral hydroxylamine (HA), which  
149 specifically cleaves thioesters but not oxygen esters or amides (9). HA treatment of cells producing the  
150 Lnt(C23,C62A) double mutant quantitatively liberated the C387 thiol, as shown by malPEG



Thioester acyl-Lnt intermediate

151 modification (Fig. 3). Lnt lacking all cysteines (**Lnt(C23,C62AC387S)**) was not modified by malPEG  
152 after HA treatment . These data indicate that the extra-cytoplasmic catalytic thiol of Lnt exists in a  
153 thioester acyl enzyme intermediate *in vivo*.

154  
155 *Lnt(C23,C62AC387S) forms an oxygen-ester acyl enzyme intermediate.*

156 To demonstrate directly that Lnt was acylated on C387, cells induced for synthesis of Lnt variants  
157 with different substitutions at C387 were labeled with <sup>3</sup>H-palmitate and analyzed by fluorography. <sup>3</sup>H-  
158 palmitate was incorporated into both Lnt(C23,C62A) and **Lnt(C23,C62AC387S)**, but not  
159 Lnt(C23,C62,C387A) (Fig. 4). The thioester formed at C387 with the labeled palmitate proved to be  
160 sensitive to neutral HA, both in terms of losing the label and becoming sensitive to derivitization with  
161 malPEG, whereas the C387S label did not. These results indicate that C387 indeed forms a thioester  
162 bond with palmitate and also that, if the conserved Cys is replaced by Ser, Lnt is able to form an  
163 oxygen-ester that is resistant to HA treatment. Furthermore, only residue 387 is involved in the  
164 formation of acyl enzyme intermediates, since **Lnt(C23,C62,C387A)** does not incorporate fatty acids .

165  
166 *Residues affecting thioester acyl enzyme formation and N-acylation of apolipoproteins.*

167 Previously, several essential residues in Lnt were identified by site-directed mutagenesis and an *in*  
168 *vivo* complementation assay (5). To examine whether the defects in these mutants could be correlated  
169 with thio-acylation of Lnt, each mutation was created in the Lnt(C23,C62A) background and the effect  
170 on the state of the C387 thiol determined by malPEG alkylation. Lnt variants were also analyzed for  
171 maleimide modification after HA treatment as a control for the accessibility of the thiol group of C387.  
172 A double band was detected in **Lnt(C23,C62AC387S)**, probably representing non-acylated Lnt (lower  
173 band) and oxygen-ester acyl-Lnt (upper band) (Fig. 5). This Lnt variant was not modified by malPEG  
174 because it lacks C387. Lnt(C23,C62A) was modified by malPEG only after treatment with HA as seen  
175 before (Figs. 3, 4 and 5). Lnt variants with substitutions at two glutamate residues E267 and E343

Thioester acyl-Lnt intermediate

176 exhibited malPEG modification independent of HA treatment, indicating that both mutations cause a  
177 defect in thio-acylation of C387 (Fig. 5). These residues therefore play a role in the first step of the *N*-  
178 acyltransferase reaction. Lnt containing the K335A substitution is partially alkylated by malPEG in the  
179 absence of HA, suggesting that it plays a role in the stabilization of the tetrahedral intermediates that  
180 are formed as part of both steps (10). The W237, Y388 and E389 substitutions showed no malPEG  
181 alkylation (or slight modification in case of W237) in the absence of HA, indicating that C387 is  
182 acylated in these allele products . These substitutions affect the maturation of major lipoprotein Lpp (5)  
183 and are thus required for the *N*-acylation of substrate apolipoproteins.

184

## 185 **DISCUSSION**

186

187 Based on similarities of the Lnt periplasmic domain with the nitrilase superfamily, it has been  
188 proposed that the apolipoprotein *N*-acylation involves a thioester acyl enzyme intermediate between an  
189 acyl group derived from 1-*sn*-glycerolphospholipid, usually phosphatidylethanolamine (PtdEtn) *in vivo*  
190 (11), and the thiol of C387 (see scheme 1). Here we have presented evidence supporting this model,  
191 showing that *in vivo*, the bulk of Lnt molecules exist as the C387-acyl-enzyme intermediate.

192 To our knowledge, Lnt is one of the few enzymes that exists *in vivo* in its reaction intermediate form  
193 and is the only protein found to have a persistent extracytoplasmic thioester of any kind. This suggests  
194 that the thioester linkage is sequestered from the aqueous milieu of the periplasm, presumably by  
195 having the C387 at the membrane interface, which might also facilitate the thermodynamically  
196 unfavorable loading reaction where the *sn*-1 acyl group of linked in oxygen-ester to  
197 phosphatidylethanolamine is transferred to the C387 thiol. Since each of the  $\sim 10^6$  lipoproteins  
198 produced in the bacterial cell per generation must be processed by Lnt, the total flux of fatty acid  
199 through the Lnt thio-ester must be upwards of  $2 \times 10^4$  molecules/min. Thus the persistent thio-ester  
200 form of Lnt is likely to be in a state of rapid synthesis and discharge into nascent lipoproteins.

#### Thioester acyl-Lnt intermediate

201 Nevertheless, the persistent intermediate state of Lnt stands in distinct contrast to Sortase, which  
202 catalyzes the covalent attachment of secreted proteins carrying the LPXTG motif to the peptidoglycan  
203 of Gram-positive bacteria, and which has also been shown to use a covalent thiol intermediate (12).  
204 However, probably due to its transient character, the thioester between sortase and LPXTG-containing  
205 surface proteins has not been directly demonstrated *in vivo*. Suree *et al.* recently reported the solution  
206 structure of sortase with a covalently bound peptide analog of the LPXTG sorting signal (13). The  
207 attack of the carbonyl group of threonine by the active site cysteine leads to the formation of a thioester  
208 and results in structural changes in the overall sortase structure. This movement creates an entry point  
209 for the peptide portion of lipid II.

210 The first step of the *N*-acyltransferase reaction requires the active site residues C387 and E267 as  
211 well as E343. In the predicted structure of the nitrilase domain of Lnt, E343 is located on a flexible  
212 loop facing away from the active site (5). The data presented here suggest a role for E343, together  
213 with E267, in the activation of C387 for attack on the carbonyl group of the phospholipid.  
214 Alternatively, this residue might be involved in positioning the phospholipid in close proximity of the  
215 active site pocket to facilitate nucleophilic attack by C387. The acyl moiety at the 1-position of PtdEtn  
216 is the acyl donor for Lnt (11). Electrostatic interactions between the positively charged head group of  
217 PtdEtn and the negatively charged E343 could therefore stabilize the interaction between phospholipid  
218 and Lnt. Phosphatidylglycerol (PtdGro) and cardiolipin (CL) can also act as acyl donors for Lnt in  
219 strains lacking the phosphatidylserine synthase gene (*pss*) (14). The intermediate state of Lnt has not  
220 been examined under these conditions.

221 In the second step of the reaction, the acyl group is transferred to the  $\alpha$ -amino group of  
222 apolipoprotein. The active site residue C387 is required, as are W237, Y388 and E389. Like E343,  
223 W237 faces away from the active site pocket. We hypothesize that W237 and Y388 recognize and  
224 interact with the diacylglyceryl moiety of C<sub>+1</sub> of apolipoprotein. Five of the seven essential residues are  
225 conserved in Lnt of *M. smegmatis*, but W237 and Y388 are altered (2). Mycobacterial lipoproteins are

Thioester acyl-Lnt intermediate

226 modified with a diacylglyceryl containing mycobacterial specific fatty acids. The fact that Lnt of *M.*  
227 *smegmatis* cannot complement a conditional *E. coli lnt* mutant suggests that W237 and Y388 play a  
228 role in substrate specificity. In *Mycobacteria*, *lnt* is found in an operon (*M. smegmatis*) or fused (*M.*  
229 *tuberculosis*) with a gene (*ppm-1*) encoding polyprenol monophosphomannose synthase (15). The *N*-  
230 acyltransferase domain enhances Ppm-1 activity but the effect of mannosyltransferase on Lnt function  
231 is currently unknown (15). K335 probably stabilizes the oxyanion of the tetrahedral intermediates that  
232 are formed as part of the Lnt reaction similar to the reaction catalyzed by members of the nitrilase  
233 family. Apolipoprotein fulfills hereby the role of a water molecule in the second step of the Lnt  
234 reaction (16).

235 Lnt forms an oxygen-ester acyl enzyme intermediate when the thiol group of cysteine is replaced  
236 with the hydroxyl group of serine. This enzyme is not functional because it is unable to donate the acyl  
237 group to apolipoprotein. Whether E267 is required for the acylation of serine is unknown.

238 In the structural model, E343 and W237 are located on loops facing away from the active site pocket  
239 (5) that are predicted to be flexible and to open and close upon phospholipid and substrate binding,  
240 respectively. The formation of the thioester enzyme probably involves structural rearrangements of the  
241 active site allowing entry of the apolipoprotein substrate, similar to sortase. Alternatively, the loops  
242 may be closely located to the outer leaflet of the inner membrane in order to cap the active site pocket  
243 on top of the phospholipid bilayer, there by allowing easy access to the 1-carbonyl group of PtdEtn in  
244 the first step (E343) and positioning of the diacylglyceryl cysteine for acylation in the second step  
245 (W237).

246

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249

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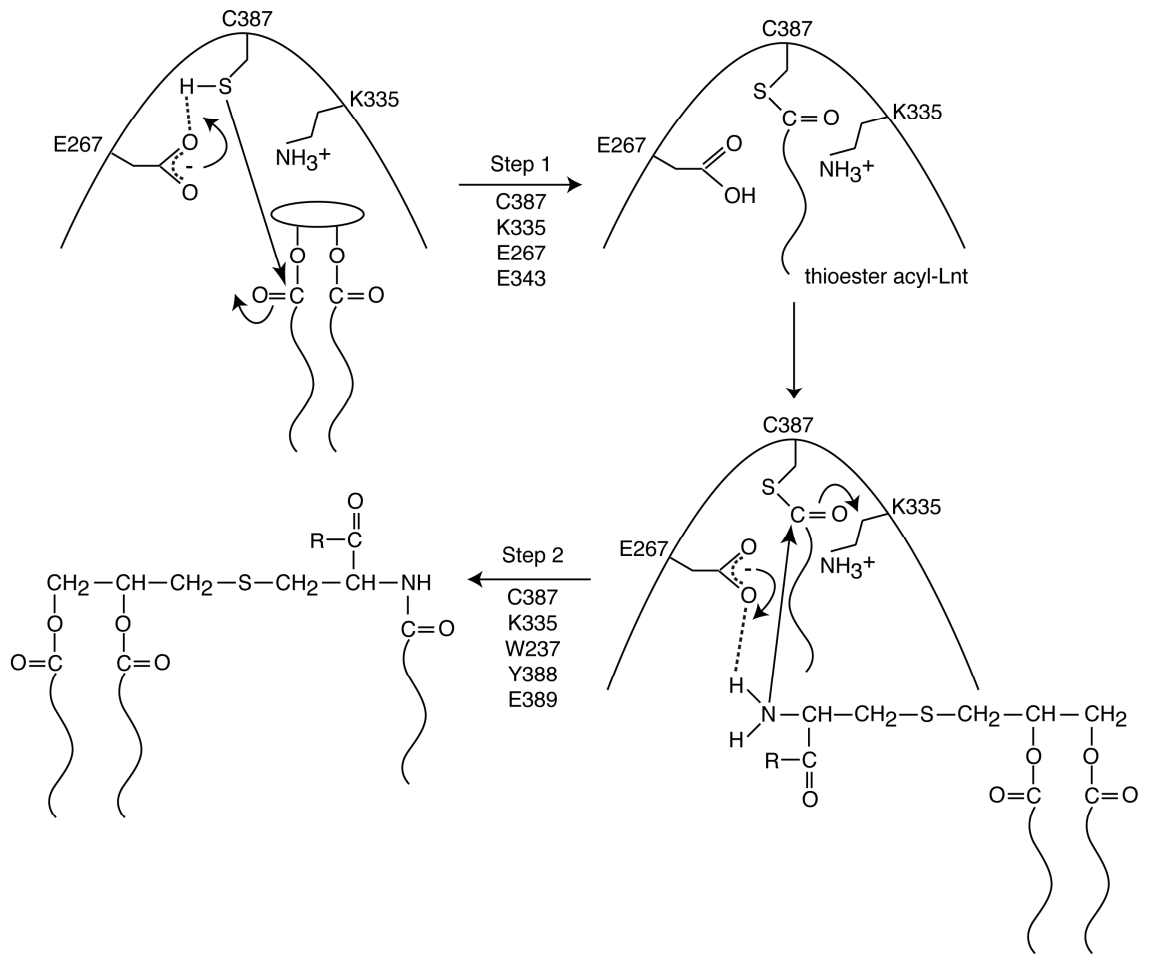
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Thioester acyl-Lnt intermediate

Scheme 1



305

306



Thioester acyl-Lnt intermediate

307 **FIGURE LEGENDS**

308

309 **Figure 1 : Lipoprotein modification in Gram-negative bacteria**

310 a. Maturation of tri-acylated lipoproteins is subsequently catalyzed by  
311 phosphatidylglycerol::prolipoprotein diacylglyceryl transferase (Lgt), prolipoprotein signal peptidase  
312 (LspA) and apolipoprotein *N*-acyltransferase (Lnt). b. Membrane topology of Lnt in the cytoplasmic  
313 membrane of *E. coli* (17). Native cysteine residues are indicated, C387 is located in the nitrilase  
314 domain (residues 220-468) facing the periplasm. The catalytic triad residues E267-K335-C387 are  
315 illustrated as rectangles, other essential residues W237, E343, Y388 and E389 are indicated as circles.  
316 Charged residues surrounding the transmembrane segments are shown with – and + symbols.

317

318 **Figure 2 : Lnt is blocked from alkylation by malPEG**

319 Cells producing variants of Lnt were treated with alkylating reagent malPEG after sample denaturation  
320 with CHCl<sub>3</sub>/MeOH. Gene expression was induced with 1 mM IPTG using pUC18-*lnt* derivatives.  
321 Equal amounts of protein were analyzed on an immunoblot that was developed with antibodies against  
322 c-Myc. Non-modified Lnt (Lnt), alkylated Lnt (Lnt-malPEG) and Lnt dimer (\*) are indicated. The  
323 dimeric form of Lnt is dependent on C23 and can be reduced in the presence of reducing agents (not  
324 shown).

325

326 **Figure 3 : Lnt is alkylated by malPEG after treatment with neutral hydroxylamine**

327 Total extracts of cells producing Lnt with C387 as the only cysteine residue Lnt(C23,C62A) or Lnt  
328 lacking all three cysteines **Lnt(C23,C62AC387S)** were alkylated with malPEG after treatment with  
329 neutral hydroxylamine. Samples were separated by SDS-10%PAGE and analyzed by immunoblotting  
330 with antibodies against c-Myc.

331

Thioester acyl-Lnt intermediate

332 **Figure 4 : Lnt forms thioester acyl enzyme intermediate**

333 a. Labeling of Lnt(C23,C62A) and **Lnt(C23,C62AC387S)** with <sup>3</sup>H-palmitate. (-) vector only sample.  
334 Gene expression was induced from the p<sub>ara</sub> promoter of pBAD18-*lnt* derivatives with 0.2% L-  
335 arabinose. Whole cells were treated with neutral hydroxylamine to release <sup>3</sup>H-palmitate from thioester  
336 acyl enzyme linkage (lanes 2, 4 and 6). b. **Lnt(C23,C62C387A)** does not incorporate <sup>3</sup>H-palmitate (lane  
337 5). Lnt(C23,C62A) and **Lnt(C23,C62AC387S)** were labeled in parallel and served as controls. c.  
338 Release of <sup>3</sup>H-palmitate was verified by alkylation with malPEG and analysis on immunoblot using  
339 antibodies against c-Myc. Lnt(C23,C62A) was alkylated by malPEG after treatment with HA (lane 3)  
340 but **Lnt(C23,C62AC387S)** was not (lane 7).

341  
342 **Figure 5 : Effect of essential residues on the acylation state of Lnt**

343 Seven substitutions that abolished function were combined in Lnt(C23,C62A) and analyzed for Lnt  
344 acylation by malPEG alkylation. **Lnt(C23,C62AC387S)** lacks all cysteines and is included as a control.  
345 Whole cells were treated (+) or not (-) with 1M neutral hydroxylamine and subsequently with malPEG.  
346 Samples were separated by SDS-10%PAGE and analyzed by immunoblotting with antibodies against  
347 c-Myc. Alkylated (Lnt-malPEG) and non-alkylated forms of Lnt are indicated.

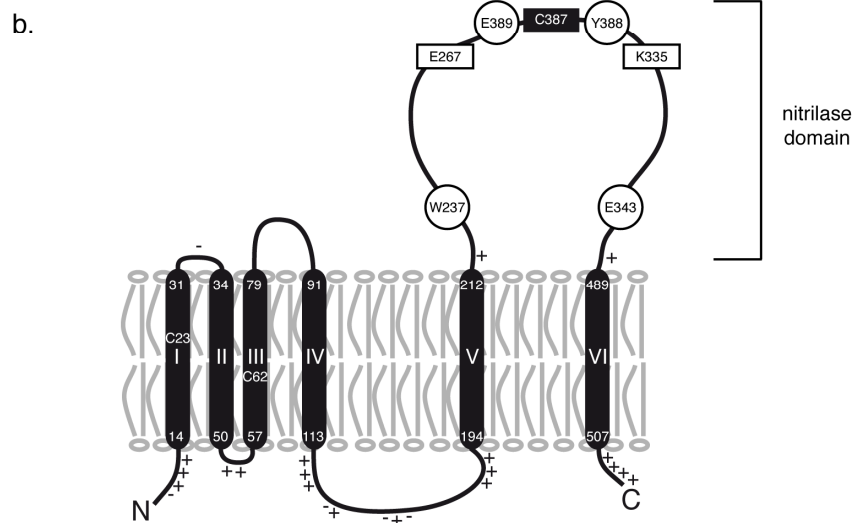
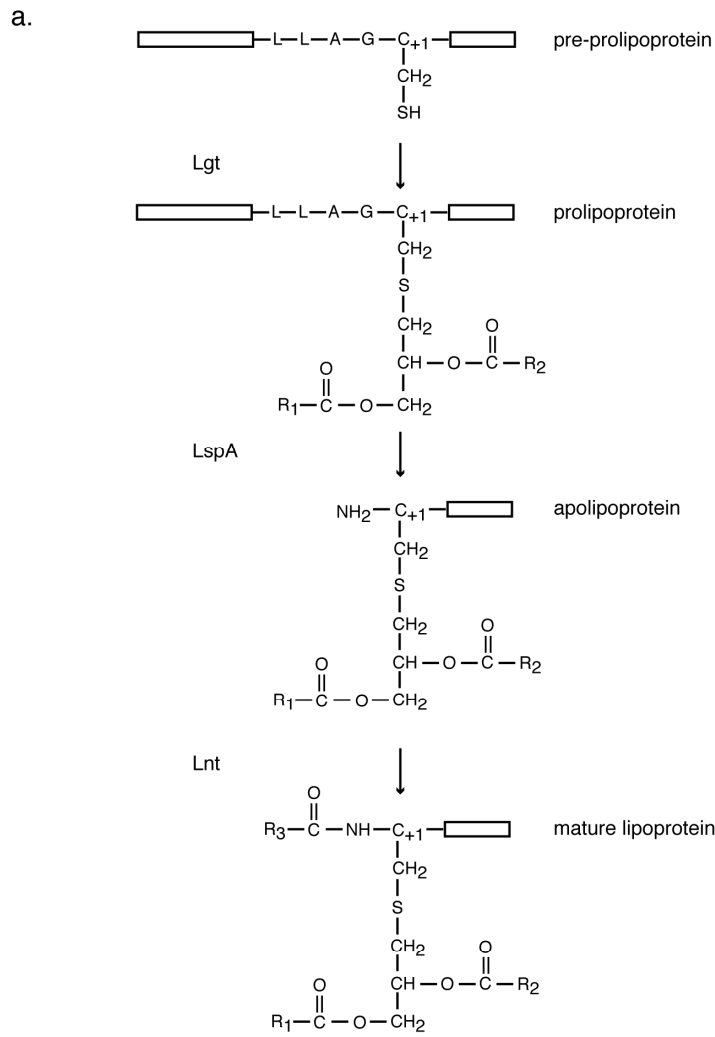
348  
349 **Scheme 1: Model for N-acyltransferase reaction and the role of essential residues**

350 Model of the Lnt reaction. Step 1 : formation of the thioester acyl enzyme intermediate. E267 activates  
351 the sulfhydryl group of C387 that can then attack the 1-carbonyl-group of phospholipids. Besides the  
352 active site residues C387 and E267, residues E343 and K335 are also required. Step 2 : formation of *N*-  
353 acyl-*S*-diacylglyceryl (mature) lipoprotein. W237, Y388, E389 and the active site residues C387 and  
354 K335 are necessary for this step. Tetrahedral intermediates are formed as part of the reaction; in step 1  
355 between Lnt and 1-acyl group of phospholipids, in step 2 between Lnt and apolipoprotein (not shown).

356

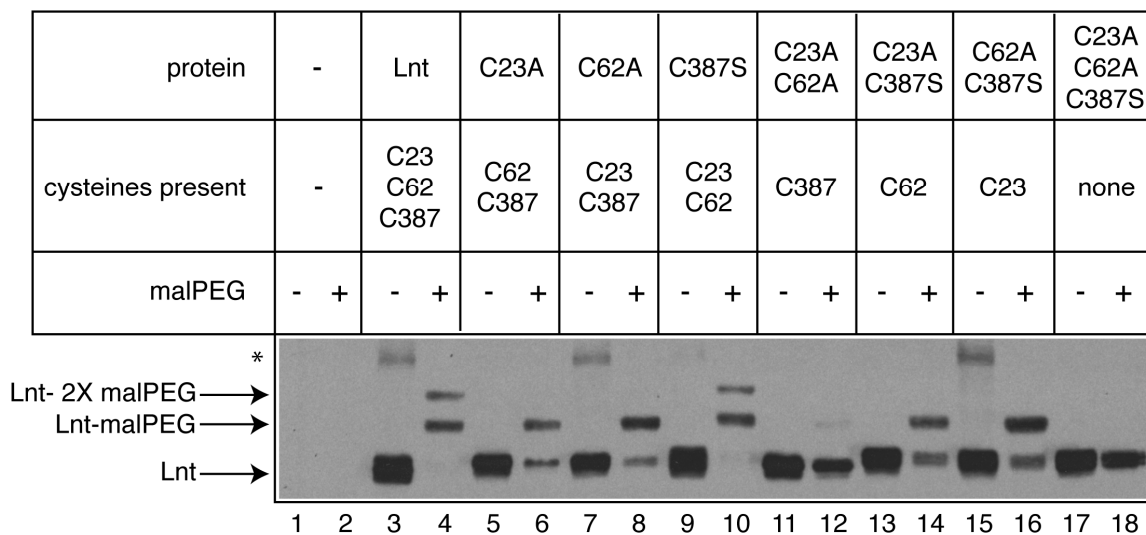
Thioester acyl-Lnt intermediate

Figure 1



Thioester acyl-Lnt intermediate

Figure 2

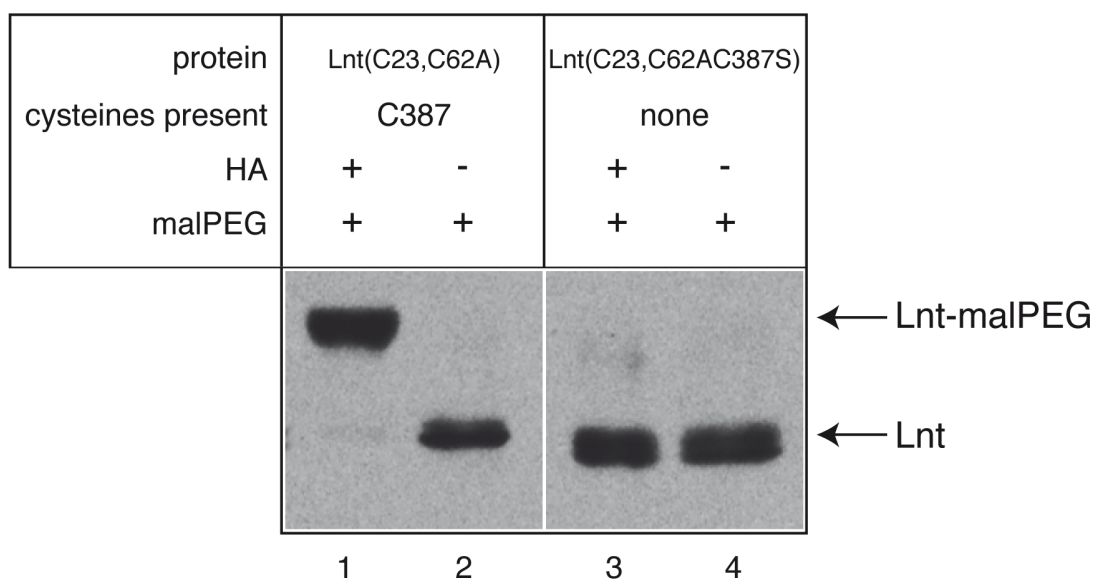


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359

Thioester acyl-Lnt intermediate

Figure 3



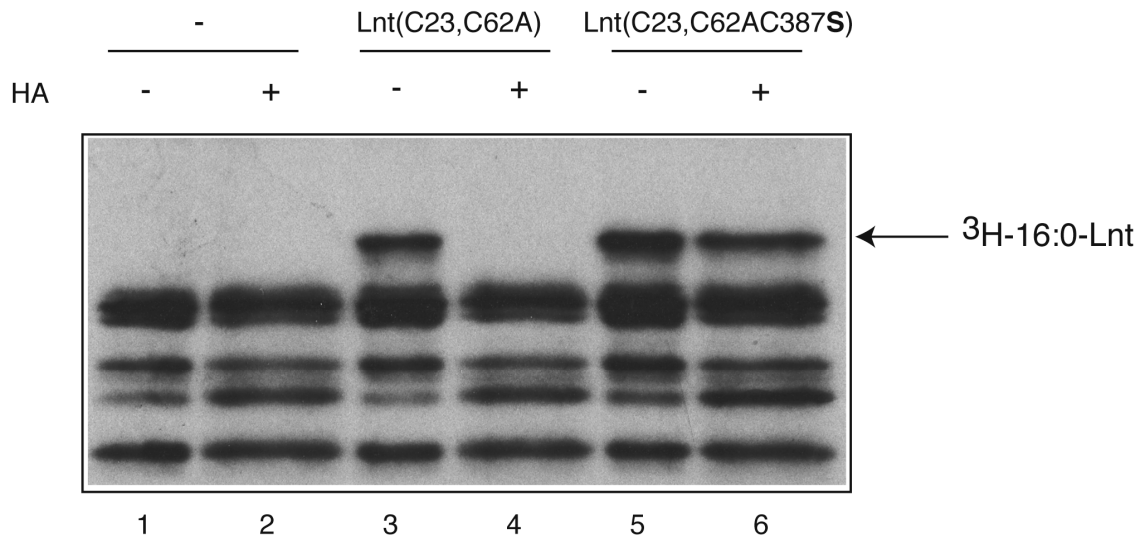
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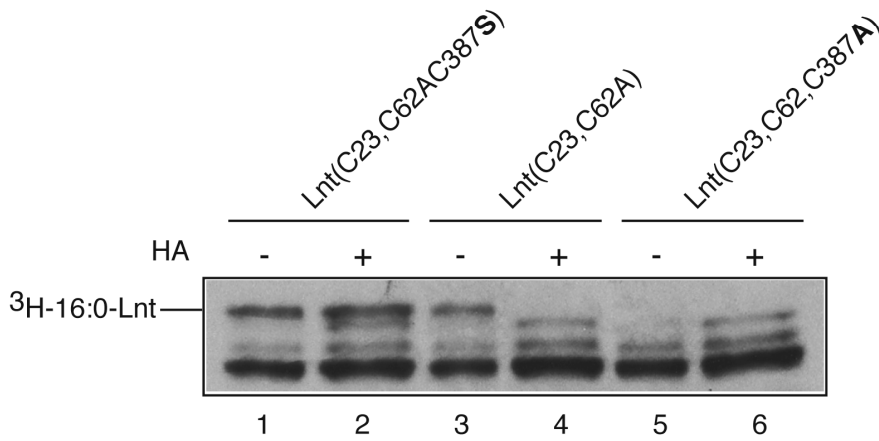
Thioester acyl-Lnt intermediate

Figure 4

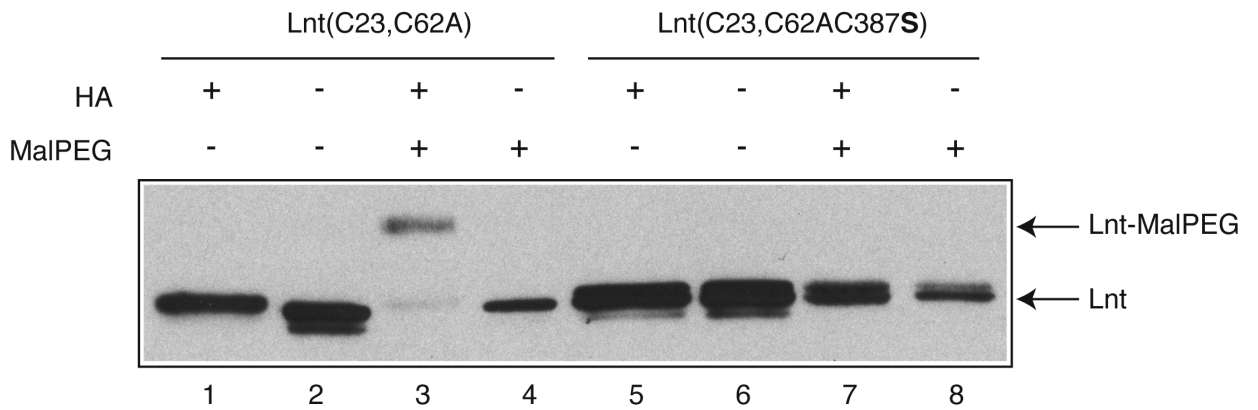
a.



b.



c.

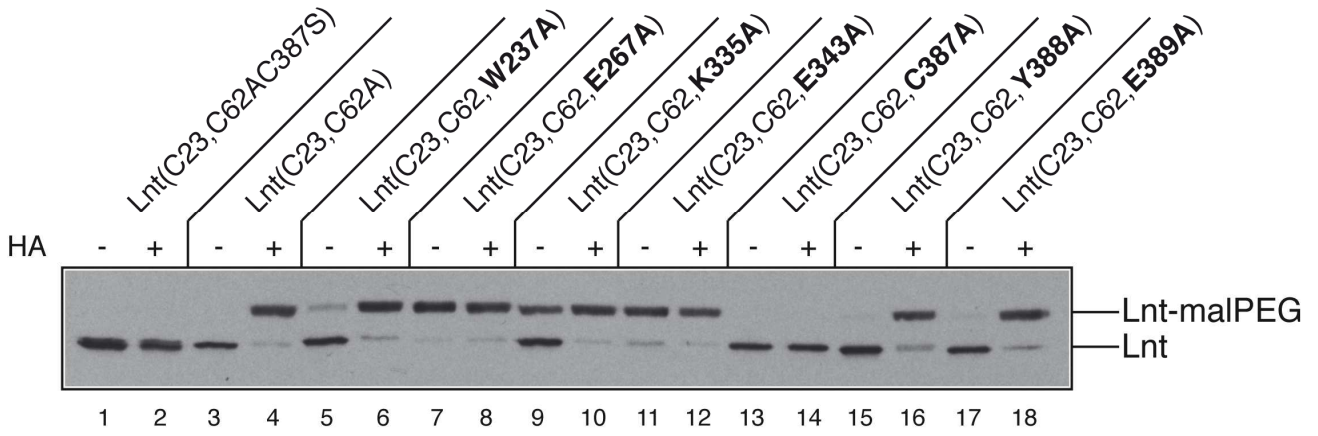


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363

Thioester acyl-Lnt intermediate

Figure 5



364

365

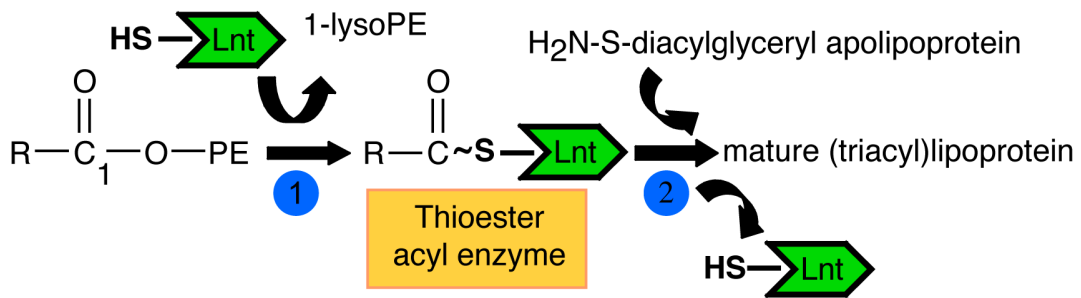
Thioester acyl-Lnt intermediate

366 THE ESSENTIAL *E. COLI* APOLIPOPROTEIN N-ACYLTRANSFERASE (LNT) EXISTS AS AN  
367 EXTRACYTOPLASMIC THIOESTER ACYL-ENZYME INTERMEDIATE

368 Nienke Buddelmeijer and Ry Young

369

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