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Molecular Characterization of the *oafA* Locus Responsible for Acetylation of *Salmonella typhimurium* O-Antigen: OafA Is a Member of a Family of Integral Membrane Trans-Acylases

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Lipopolysaccharide (LPS) coats the surface of gram-negative bacteria and serves to protect the cell from its environment. The O-antigen is the outermost part of LPS and is highly variable among gram-negative bacteria. Strains of *Salmonella* are partly distinguished by serotypic differences in their O-antigen. In *Salmonella typhimurium*, the O-antigen is acetylated, conferring the O5 serotype. We have previously provided evidence that this modification significantly alters the structure of the O-antigen and creates or destroys a series of conformational epitopes. Here we report the detailed mapping, cloning, and DNA sequence of the *oafA* gene. The locus contains one open reading frame that is predicted to encode an inner membrane protein, consistent with its role in modification of the O-antigen subunit. The OafA protein shows homology to proteins in a number of prokaryotic and one eukaryotic species, and this defines a family of membrane proteins involved in the acylation of exported carbohydrate moieties. In many of these instances, acylation defines serotype or host range and thus has a profound effect on microbe-host interaction.

Lipopolysaccharide (LPS) forms the outer surface of gram-negative bacteria and protects the cell from the environment. LPS is composed of lipid A, core oligosaccharide, and O-antigen (23). The O-antigen polymer of *Salmonella enterica* serovar Typhimurium (*Salmonella typhimurium*) LPS has a branched-tetrasaccharide repeating subunit with galactose-rhamnose-mannose in the main chain and abequose attached to the mannose residue (13). The 2-hydroxyl group of the abequose moiety is acetylated, conferring the O5 serotype (13). We have previously characterized a series of monoclonal immunoglobulin A antibodies directed against *S. typhimurium* O-antigen and have shown that the epitopes of all of these antibodies are affected by this acetylation reaction. We proposed that the acetylation affected the three-dimensional structure of the molecule and that many of the antibodies recognize conformational epitopes that are created or destroyed depending on the acetylation state of the molecule (28).

In pathogenic bacteria, O-antigen is clearly important for infection. It is the dominant antigen in many immune responses and has been shown in a number of species to be critical for protection (see reference 20 for a review). Indeed, we have shown that one of our monoclonal immunoglobulin A antibodies is capable of protecting mice against oral infection with *S. typhimurium* and that this protection was completely dependent on the acetylation and therefore on the conformation of the O-antigen (19, 28).

The acetylation reaction is dependent on the *oafA* locus (13, 28), which was originally mapped by Hfr crosses between naturally O5-positive and O5-negative strains of *Salmonella* (14). Here we report the detailed mapping, cloning, and DNA sequence analysis of the *oafA* gene. The results suggest that a single protein is responsible for the acetylation reaction. The putative OafA protein shows homology to a number of proteins involved in acylation of sugar moieties in a variety of prokaryotic and one eukaryotic species, and this defines a family of inner membrane trans-acylases.

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MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used in this study are listed in Table 1. Plasmids pJS203, pJS205, pJS206, pJS211, pJS214, pAL13, and pAL16 are derivatives of pWKS30 (31) containing *S. typhimurium* chromosomal inserts from the region around the *oafA* locus. The extent and structure of these inserts are indicated in Fig. 1. The plasmid pWKS30 is a low-copy pSC101-based plasmid that confers ampicillin resistance. It contains a polycloning site adjacent to a T7 RNA polymerase promoter, all located within a *lacZ*α gene (31). P22 and P1 transductions were carried out as described elsewhere (18, 26).

Plasmid construction and isolation. Total chromosomal DNA was isolated from MT120 (28) and digested with *Eco*RI. The resulting fragments were ligated into the *Eco*RI site of pUC19 (34). The ligation mixture was electroporated into *Escherichia coli* DH5α and plated on Luria-Bertani (LB)-kanamycin, thus selecting for a clone that contains the *oaf-127*::Tn*10d*-Km insertion mutation carried on an *Eco*RI chromosomal fragment. (There are no *Eco*RI sites in Tn*10d*-Km [15]). The resulting plasmid was named pMM68. Its structure was confirmed by restriction analysis.

In order to remove Tn*10d*-Km from the plasmid by recombination, pMM68 was electroporated into wild-type *S. typhimurium* 14028, and isolated strains containing the plasmid were grown overnight in LB-ampicillin. Plasmid DNA was isolated and used to electroporate DH5α. Electroporated cells were plated on LB-ampicillin and subsequently replica plated to LB-ampicillin-kanamycin. Plasmids that had lost the Tn*10d*-Km insertion were identified by their Ap^r Km^s phenotype. One clone was purified, and the resulting plasmid was designated pJS200. The structure of the plasmid was confirmed by restriction analysis. The chromosomal insert in pJS200 was isolated and cross-linked to horseradish peroxidase according to the supplier's instructions (Amersham). This horseradish peroxidase-conjugated DNA was used to probe a previously constructed *S. typhimurium* chromosomal library. This library contains 6- to 10-kb chromosomal DNA fragments from *S. typhimurium* 14028 in the low-copy cloning vector pWKS30. Four plasmids that hybridized with the chromosomal insert in pJS200 were isolated.

The plasmids isolated above were restriction mapped. In addition, each was electroporated into *S. typhimurium* 14028, MT96, and MT120 in order to test their ability to complement the *oafA* insertion mutations. The 4-kb *Kpn*I frag-

TABLE 1. Bacterial strains and plasmids

Bacterial strain or plasmid	Genotype or description	Reference or source
Strains		
S. typhimurium		
14028	Wild type	ATCC[a]
MT96	14028 *oafA126*::Tn*10d*-Tc	28
MT120	14028 *oafA127*::Tn*10d*-Km	28
JS121	14028 *galE496 oafA127*::Tn*10d*-Km	This study
JS122	14028 *galE496 zei8102*::Mu*d*Cm *fruB124*::Tn*10*	This study
JS123	14028 *galE496 oafA127*::Tn*10d*-Km *zei8102*::Mu*d*Cm	This study
JS124	14028 *galE496 fruB124*::Tn*10*	This study
JS125	14028 *galE496 ompC396*::Tn*10*	This study
JS126	14028 *galE496 ompC396*::Tn*10 oafA127*::Tn*10d*-Km	This study
JS127	14028 *galE496 zei8102*::Mu*d*Cm	This study
TN3740	LT2 *leu-485 trp190*::(*lac*I *lacp* T7*pol spc*)	Charles G. Miller
E. coli		
AL13	F′42 *lac*$^+$ *finP301/endA1 hsdR17 supE44 thi recA1 gyrA96 relA1 lac*	This study
DH5α	F$^-$ *endA1 hsdR17 supE44 thi-1 recA1 gyrA relA1* Δ(*lac-argF*)*U169 deoR* [ϕ80d*lac*Δ(*lacZ*)*M15*]	33
JMS1160	F$^-$ *araD139* Δ(*lac-argF*)*U169 rpsL150 relA flb5301 ptsF25 deoC1 recA111*::Km	29
Plasmids		
pUC19	*colE1* Ap^r	34
pMM68	pUC19 *oafA127*::Tn*10d*-Km	This study
pJS200	pMM68 that has lost Tn*10d*-Km	This study
pWSK30	pSC101 derivative Ap^r	31
pJS208	pJS205 *oafA126*::Tn*10d*-Tc	This study

[a] ATCC, American Type Culture Collection.

ment from pJS205 (one *Kpn*I site is in the multiple cloning site of pWKS30) was isolated and cloned into the *Kpn*I site of pWSK30, yielding pJS211.

The position of the *oaf-127*::Tn*10d*-Km insertion was determined by DNA sequence analysis of pMM68 using primers specific for the ends of the Tn*10d*-Km element. In order to determine the location of the *oaf-126*::Tn*10d*-Tc, pJS205 was electroporated into MT96 and an Ap^r clone was grown overnight in LB-ampicillin. Plasmid DNA was isolated and used to electroporate DH5α, and Tc^r clones were selected. One of the plasmids, pJS208, corresponded to pJS205 into which the Tn*10d*-Tc insertion had recombined. The relative position of the insertion on the chromosomal fragment was determined by restriction analysis.

Agglutination reactions. Agglutination of whole bacterial cells with monoclonal antibody Sal4 was performed in round-bottom microtiter dishes. Cells were grown overnight in LB. One milliliter of overnight culture was centrifuged, and the cells were resuspended in 1 ml of phosphate-buffered saline (PBS). Then 100 μl of cells was mixed with 100 μl of a 1/40 dilution of Sal4 ascites fluid in PBS and incubated at room temperature for 3 to 4 h.

DNA sequence analysis. Most of the DNA sequence was obtained with primers specific to the ends of the insertion element γδ (10). Multiple independent γδ insertions were isolated in pJS211 by conjugal transfer from strain AL13 containing the plasmid to JMS1160, selecting for Ap^r Kn^r colonies. In addition, several primers were synthesized to allow sequencing of small regions not obtained in sequencing the γδ insertions. DNA sequencing was performed by the Genetic Engineering Facility at the University of Illinois Biotechnology Center.

Nucleotide sequence accession number. The *oafA* sequence has been entered into GenBank under accession number U65941.

RESULTS AND DISCUSSION

Genetic mapping of the *oafA* locus. We have previously isolated two independent insertion mutations in the *oafA* locus of *S. typhimurium* that prevent acetylation of O-antigen and

TABLE 2. Results of three-factor P1 crosses to map *oafA*

Cross	Characteristics of strain[a]		Marker (no. of transductants [%])	
	Donor	Recipient	Selected	Unselected
1	*oafA127*::Tn*10d*-Km	*zei8102*::Mu*d*Cm *fruB124*::Tn*10*	Km^r (150 [100])	Cm^r Tc^r (80 [53.3]) Cm^s Tc^s (65 [43.3]) Cm^s Tc^r (4 [2.7]) Cm^r Tc^s (1 [0.7])
2	*oafA127*::Tn*10d*-Km *zei8102*::Mu*d*Cm	*fruB124*::Tn*10*	Km^r (150 [100])	Cm^s Tc^r (79 [52.7]) Cm^r Tc^s (58 [38.7]) Cm^r Tc^r (13 [8.7]) Cm^s Tc^s (0 [0])
3	*ompC396*::Tn*10*	*oafA127*::Tn*10d*-Km *zei8102*::Mu*d*Cm	Tc^r (150 [100])	Km^r Cm^r (80 [53.3]) Km^s Cm^r (51 [34.0]) Km^s Cm^s (17 [11.3]) Km^r Cm^s (2 [1.3])
4	*ompC396*::Tn*10 oafA127*::Tn*10d*-Km	*zei8102*::Mu*d*Cm	Tc^r (150 [100])	Km^s Cm^r (105 [70.0]) Km^r Cm^r (41 [27.3]) Km^r Cm^s (4 [2.7]) Km^s Cm^s (0 [0])

[a] The strains used were as follows: cross 1, JS121 (donor) and JS122 (recipient); cross 2, JS123 (donor) and JS124 (recipient); cross 3, JS125 (donor) and JS123 (recipient); cross 4, JS126 (donor) and JS127 (recipient).

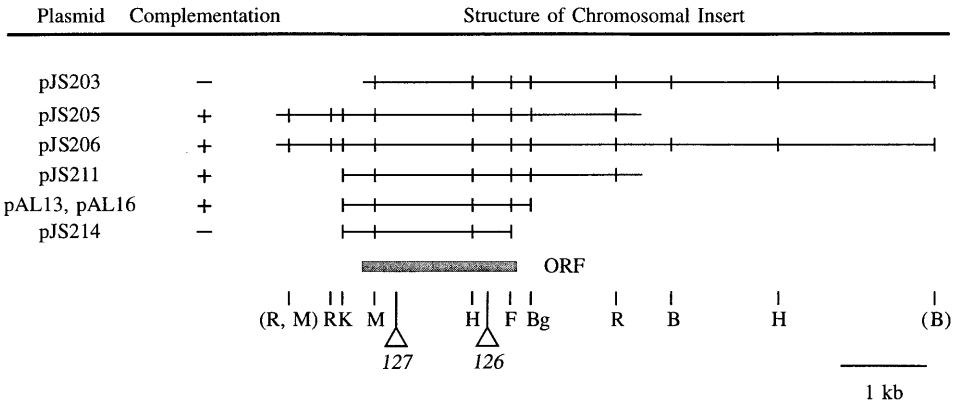

FIG. 1. Localization of the *oafA* locus. The chromosomal inserts in various pWKS30 derivative plasmids are indicated. R, *Eco*RI; M, *Mlu*I; K, *Kpn*I; H, *Hin*dIII; F, *Fsp*I; Bg, *Bgl*I. The left and right ends of pJS203, pJS205, and pJS206 are delineated by *Sau*3AI sites cloned into the *Bam*HI site in pWKS30. Therefore, it is not clear whether the site at the right end of pJS203 and pJS206 is a *Bam*HI site or simply a *Sau*3AI site in the chromosome. The order of the *Eco*RI and *Mlu*I sites at the left end of pJS205 and pJS206 has not been determined. Complementation is defined as the ability of the plasmid to confer recognition by monoclonal antibody Sal4 to both MT96 (*oaf-126*::Tn*10d*-Tc) and MT120 (*oaf-127*::Tn*10d*-Km) as measured by agglutination. The approximate location of the two insertion mutations is indicated. Also shown is the position of the putative OafA open reading frame (ORF).

therefore prevent the synthesis of the epitope recognized by a monoclonal immunoglobulin A antibody, Sal4 (28). These insertion mutations are designated *oaf-126*::Tn*10d*-Tc and *oaf-127*::Tn*10d*-Km (28). The original mapping of *oafA* placed the locus on the far side of *metG* (min 47) from *rfb* (min 45) on the *S. typhimurium* chromosome (14, 25). We previously reported Hfr data that placed *oafA* near *glpQTA* at min 49 (28). We have now mapped *oafA* more precisely. The *oafA* locus is 1% linked by P22 transduction to *fruB* (min 48.1) but is not linked by P22 transduction to either *ompC*, *gyrA*, or *glpQTA* (all at min 49). In order to further clarify the position of *oafA* with respect to *fruB* and *ompC*, we performed three-factor crosses using *S. typhimurium* strains which were *galE* mutants and therefore P1 sensitive. Because P1 packages 90 kb of DNA whereas P22 packages 45 kb of DNA, P1 can be used to map loci that are unlinked by P22 transduction. Both the *fruB* and *ompC* mutations were the result of Tn*10* insertions. Therefore, we first isolated a Mu*d*Cm insertion (*zei8102*::Mu*d*Cm) that is 85% linked to *fruB* and 10% linked to *oafA* by P22 transduction. P1 transductional mapping and reciprocal three-factor crosses were then used to map the relative positions of the various markers. The results (Table 2) show unambiguously that the gene order is *fruB zei8102*::Mu*d*Cm *oafA ompC*. Thus, *oafA* is located between *fruB* and *ompC* at min 48.5 on the *S. typhimurium* chromosome. We also have evidence showing that the *oafA* locus is transcribed clockwise on the *S. typhimurium* chromosome toward *ompC* (27).

Cloning of the *oafA* locus. In order to characterize the *oafA* locus in greater detail, we cloned the *oaf-127*::Tn*10d*-Km insertion mutation on an *Eco*RI fragment, yielding pMM68. A derivative of pMM68 was then used as a probe to isolate several plasmids from a low-copy plasmid library of our wild-type *S. typhimurium* ATCC 14028. The chromosomal inserts in several of the isolated plasmids are shown in Fig. 1. Each of the plasmids was introduced into the two *oafA* insertion mutants, and production of the Sal4 monoclonal immunoglobulin A antibody epitope was determined by agglutination. The plasmids pJS205 and pJS206 complemented both insertion mutations, whereas pJS203 did not. A 4-kb *Kpn*I fragment from pJS205 was then subcloned, yielding pJS211, and a 2.2-kb *Kpn*I-*Bgl*I fragment was subcloned in both orientations to construct pAL13 and pAL16. The *Kpn*I-*Fsp*I fragment was cloned from pAL16 to yield pJS214. The plasmids pJS211, pAL13, and pAL16 complemented both *oafA* insertion mutations, whereas pJS214 did not. Taken together, these results indicate that the *oafA* locus is contained on the 2.2-kb *Kpn*I-*Bgl*I fragment.

DNA sequence analysis of *oafA*. The DNA sequence of both strands of the *Kpn*I-*Bgl*I fragment was determined. This region contains one large open reading frame corresponding to a protein of 609 amino acids. A potential promoter with good

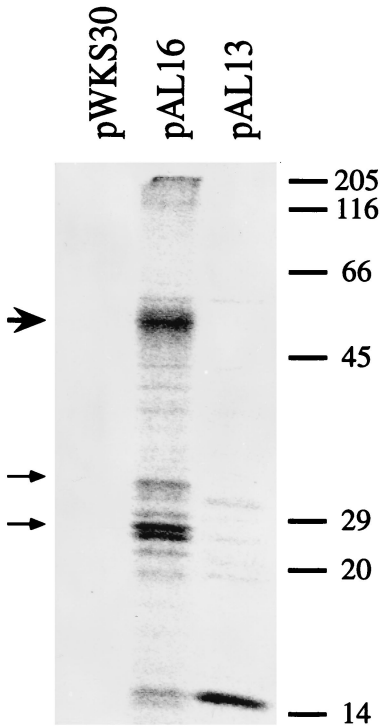

FIG. 2. Expression of OafA protein from a T7 promoter. The plasmid pAL16 contains the OafA open reading frame in the correct orientation with respect to the T7 promoter. Plasmid pAL13 contains the same fragment in the opposite orientation and pWKS30 is the parent vector. The relative positions of the molecular weight (in thousands) markers are indicated. Protein bands unique to pAL16 are indicated by arrows.

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OafA          33 GGFIGVDVFFVISGFLMT GIVLERVDHKGVLD.............. FYIARFLRIVPAL  77
Hi0392        -5 GGFLGVDIFFVISGFLIT GIIITEIQQNSFSLKQ............. FYTRRIKRIYPAF  42
Oac           40 AGGIAVIIFFSISGYLIS ..................KSAIRSDSFID FMAKRARRIFPAL  81
NodX          51 FSAPGVAIFFLISGFLVT ..................DSYIRSSSAAS FFVKRSLRIFPAL  92
ExoZ          15 IGAAGVDVFFVISGFIMW ................VISDRRSVTPVE FIADRARRIVPVY  57
PicA Orf2     38 SSYLAVDLFFALSGFVLA ............HAYGKKLYEGTITPGF FLKARFARLYPLY  84
CarE          48 LGPLTVSFFFMLSGFVLT ..................WAGLPDKSKVN FWRRRTVRAYSLH  89
Mpt           48 LGPVAV.FFFMLSGFVLT ..................WAGMPDPSKPA FWRRRWVRVYSLH  88
AcyA          48 LGSLAVSLFFVLSGYVLT ..................WSARDGDSVRS FWQRRFAKIYPLH  89
MdmB          48 LGSIAVSVFFLLSGFVLA ..................WSARDKDSVTT FWRRRFAKIYPLH  89
Lag1          45 FQSLAVNAFFWLSGFLIT ...........YHCITKKPY....TFAE YMIDRFCRIYVIY  88
T08H10.4      29 NGYIGVDMFFVLSGFLMA .........MIISSKPITWNSVYQ.... FYFRRSKRILPL.  73
R03H4.5       28 NGFLGVDIFFVISGFLMA .......NNLTNLNLLN.....VHDFLL FYYKRFRRILPLY  74
R03H4.6       28 NGFLGVDIFFVISGFLMA .......QNLSKSKLVT.....VQDFFI FYYRRFRRILPLY  74
R03H4.1       28 NGFLGVDIFFVISGFLMA .......KILTKSSLRS.....VQDITA FYFRRFRRILTLY  74
F09B9.1      304 NAFVSVDTFFVLSGLVLT YMFFKTTPK.......KKMIVNPVTWIM FYVHRYLRLTPPI 355
C06B3.2      315 NAVFSVDTFFLVSGITVA YSFFRLKPT.......TKTLKSPATWIL FYVHRYVRLTPPY 366
C08B11.4     378 QAPLAVDSFFFLSGMLAA FSFFKKTMKADPNHPPKLSAFNWQTWPM YYYKRYIRITPTY 436
W07A12.6     124 NSALGVEIFLVLSGLLAA RSWLR...KADEPFF........QHWIT FIIRRILRLAPVM 171
W07A12.7     133 NSALGVEIFLVLSGLLAA RSWLR...KADEPFF........QHWKS FIARRLLRLAPSM 180
GumF          59 AYSFHVPLFFLVSGWLAA ..................GYASRTTSLL QTITKQARGLLLP  99
Consen           NGFLGVDIFFVLSGFLMA ---------------------------- FY-RRF-RIYPL-

OafA         134 HTWSLSVEWQFYILYPLLV 152
Hi0392        99 HIWSLAVEGQYYLIFPLIL 117
Oac          138 SLWTLPLEFLCYIITGVAV 156
NodX         155 VLWTLTVELTFYLTLPMLL 173
ExoZ         106 QGWTLNFEMLFYAVFAGSL 124
PicA Orf2    147 PAWSLFNELVVNAVYARWG 165
CarE         139 VAWSLSCELFFYAMFPFLF 157
Mpt          138 VAWSLSCEMLFYAAFPFLF 156
AcyA         140 PSWSLSCEMAFYLTFPLWY 158
MdmB         140 PSWSLSCEFAFYLTFPLWY 158
Lag1         143 PLWSIAVEWWLYTLFGIAF 161
T08H10.4      78 YTWSLCVEMQFYLLVPAIF  96
R03H4.5      136 HLWSLSVEMQFYLLVPFIF 154
R03H4.6      136 HLWSLGVEMQFYLLVPFIF 154
R03H4.1      136 HLWSLSVEMQFYILAPIVF 154
F09B9.1      413 PTWYLAVDTQLYLVAPIVL 431
C06B3.2      423 PSWYLAVDTQLYLVAPILL 441
C08B11.4     489 WTWYLANDMQFHIFLMPLL 507
W07A12.6     222 YIWYLGLDMQLYMVASIFL 240
W07A12.7     231 YLWYLGLDMQLYMVAPIFL 249

Consen           -LWSLSVEMQFYLLFPILF
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FIG. 3. Homology to OafA defines a family of trans-acylation enzymes. The two particularly strong regions of homology are shown as defined by the PILEUP program with subsequent manual adjustment. A consensus sequence is shown below. Very highly conserved amino acids are in boldface. The proteins are described in Table 3. Note that the homology to OafA extends beyond the beginning of the designated open reading frames for both Hi0392 and ExoZ (not shown), suggesting that the assignment of the initiation codon for these two proteins may be incorrect. The second region of homology is not evident in the GumF protein sequence.

correspondence to the consensus Sigma 70 promoter (12) is located such that the putative start site of transcription is approximately 44 bp upstream of the initiation ATG. The fact that this fragment complements the insertion mutations independent of orientation suggests that the fragment carries its own promoter. A potential Shine-Dalgarno sequence (3) is located upstream of the initiation codon. The designation of the initiation methionine is supported by the fact that pJS203 does not complement the insertion mutations; the chromosomal insert in pJS203 came from a partial *Sau3AI* digest, and the only *Sau*3AI site between the *Kpn*I site and the *Mlu*I site is just downstream of the designated initiation codon. The carboxy terminus of the open reading frame is also required as evidenced by the fact that plasmid pJS214, missing the last 20 amino acids of the putative protein, does not complement. A potential terminator stem-loop structure is present at the end of the open reading frame. Taken together, these results suggest that the *oafA* locus is transcribed as a monocistronic message that encodes a single protein of 609 amino acids.

Hydrophobicity analysis of the putative protein sequence of OafA suggests that it is an inner membrane protein with seven to nine transmembrane domains in the amino-terminal 350 amino acids with a distinct 250-amino-acid carboxy-terminal domain that is not hydrophobic (16). The fact that OafA is an inner membrane protein is consistent with its role in acetylation of O-antigen. The O-antigen tetrasaccharide is synthesized on the cytoplasmic face of the inner membrane attached to an undecaprenol diphosphate lipid carrier (see reference 32 for a review). Presumably, OafA acts on this O-antigen monomer and acetylates the 2-hydroxyl group of the abequose moiety. We would predict that the acetyl donor is acetyl coenzyme A, although there is no direct evidence for this hypothesis. If true, this would suggest that the acetylation reaction takes place on the cytoplasmic face of the membrane prior to the translocation of the O-antigen-undecaprenol diphosphate to the outer surface of the cytoplasmic membrane where it is polymerized and/or assembled into LPS (17, 32). If, on the other hand, the acetylation reaction takes place after translocation to the outer surface of the cytoplasmic membrane, then OafA must have access to a high-energy acetyl donor in the periplasm. This seems less likely.

Expression of the OafA protein. In order to determine if the potential open reading frame is expressed, pAL13 and pAL16 were introduced into strain TN3740, which contains the T7 polymerase under the control of LacI. Thus, induction with IPTG (isopropyl-β-D-thiogalactopyranoside) allows expression from the T7 promoter carried on pWKS30 (31), the parent plasmid of pAL13 and pAL16. The orientation is such that the

TABLE 3. Description of members of integral membrane trans-acylase family

Protein name	Size (aa)[a]	Sp.	Function[b]	Reference and/or accession no.
OafA	609	*Salmonella typhimurium*	Acetylation of LPS O-antigen	U65941
Hi0392	245[c]	*Haemophilus influenzae*	?	9; U00073
Oac	333	*Shigella* phage SF6	Acetylation of LPS O-antigen	4, 30; X56800
NodX	367	*Rhizobium leguminosarum* biovar viciae strain TOM	Acetylation of Nod factor	5, 8; X07990
ExoZ	318[c]	*Rhizobium meliloti*	Acetylation of exopolysaccharide I	2, 22; X58126
PicA Orf2	191[d]	*Agrobacterium tumefaciens*	?	24; M62814
CarE	388	*Streptomyces thermotolerans*	4″-O-acylation of macrolide antibiotics	7; D31821
Mpt	388	*Streptomyces mycarofaciens*	4″-O-acylation of macrolide antibiotics	D63662
AcyA	389	*Streptomyces thermotolerans*	3-O-acetylation of macrolide antibiotics	1; D30759
MdmB	387	*Streptomyces mycarofaciens*	3-O-acetylation of macrolide antibiotics	11; M93958
Lag1	357	*Legionella pneumophila*	Acetylation of LPS O-antigen	U32118
T08H10.4	242[e]	*Caenorhabditis elegans*	?	U55368
R03H4.5	645	*Caenorhabditis elegans*	?	U50300
R03H4.6	646	*Caenorhabditis elegans*	?	U50300
R03H4.1	600	*Caenorhabditis elegans*	?	U50300
F09B9.1	657	*Caenorhabditis elegans*	?	Z49887
C06B3.2	665	*Caenorhabditis elegans*	?	Z77652
C08B11.4	790	*Caenorhabditis elegans*	?	Z46676
W07A12.6	399	*Caenorhabditis elegans*	?	Z68320
W07A12.7	490	*Caenorhabditis elegans*	?	Z68320
GumF	364	*Xanthomonas campestris* pv. campestris	Acetylation of LPS O-antigen and xanthan polysaccharide	S47286

[a] aa, amino acids.
[b] ?, unknown.
[c] The homology to OafA extends beyond the beginning of the designated open reading frames for both Hi0392 and ExoZ, suggesting that the assignment of the initiation codon may be incorrect.
[d] The sequence of the carboxy-terminal portion of PicA Orf2 is unknown.
[e] The predicted open reading frame for protein T08H10.4 in GenBank was reevaluated, and an additional exon that includes the second highly conserved domain was added. This exon corresponds to bp 22692 to 22510 in the DNA sequence.

appropriate strand that allows translation of the open reading frame should be transcribed from pAL16. Figure 2 shows that a protein with an apparent molecular mass of 55 kDa is produced from pAL16 that is not produced from pAL13 or the vector alone. The genetic data presented above suggest that the entire open reading frame is required for OafA function. The fact that the protein is apparently smaller than the predicted 69 kDa suggests that either the membrane protein runs anomalously in the gel or it is being partially degraded. Two other bands unique to pAL16 correspond to proteins of 27 and 32 kDa. The origin of these bands is not clear, although they may be degradation products.

OafA shows homology to other integral membrane transacylases. OafA is homologous to a series of proteins that define a family of membrane proteins that are involved in the acylation of carbohydrate moieties on extracytoplasmic molecules (Fig. 3 and Table 3). These proteins include O-acetyl transferase from *Shigella flexneri* bacteriophage SF6, which acetylates the bacterial O-antigen conferring new antigenic groups (4, 30); NodX from *Rhizobium leguminosarum* biovar viciae, which is responsible for acetylation of Nod factor and confers host range specificity (5, 8); ExoZ from *Rhizobium meliloti*, which is responsible for acetylation of exopolysaccharide (2, 22); and several proteins from *Streptomyces* spp. that acylate macrolide antibiotics (1, 7, 11). Acetylation of many of these substrates has a profound effect on the interaction of the bacterium with the corresponding host organism. The group also includes nine proteins from *Caenorhabditis elegans* with unknown functions. Interestingly, there are no apparent homologs in *Saccharomyces cerevisiae*, suggesting that the transacylases in *C. elegans* may be involved in cell-cell interactions. This group of proteins is apparently unrelated to a large family of cytoplasmic transacetylases that include LacA and CysE proteins of *E. coli*; NodL proteins, involved in synthesis of Nod factor in *Rhizobium* species; and chloramphenicol acetyltransferases from a variety of bacterial species (6, 21).

Although many regions of these integral membrane proteins are similar, the homology is particularly striking in the region corresponding to amino acids 33 to 77 and amino acids 134 to 152 in OafA (Fig. 3). Interestingly, the regions corresponding to amino acids 35 to 54 and 135 to 152 in OafA are predicted to be transmembrane. Whether these highly conserved regions correspond to a conformational determinant necessary for the reaction or a binding site for a common substrate (acyl coenzyme A?) is not clear.

OafA is unique among the prokaryotic members of the family of proteins in that it has a large 250-amino-acid carboxy-terminal portion that is not predicted to be in the membrane. This portion of the protein shows no significant homology to any other protein in the databases. The protein produced from plasmid pJS214 should be missing the last 20 amino acids from the native OafA protein and should replace these with 21 amino acids produced from sequences in the plasmid prior to the first stop codon in that reading frame. This construct does not complement an *oafA* mutation, suggesting that the carboxy-terminal portion of OafA is required for function. However, we cannot distinguish whether this portion is directly involved in some aspect of the acetylation reaction or is simply required for stability of the protein.

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