



Enterobacterial Common Antigen: Synthesis and Function of an Enigmatic Molecule

Ashutosh K. Rai,^a  Angela M. Mitchell^a

^aDepartment of Biology, Texas A&M University, College Station, Texas, USA

ABSTRACT The outer membrane (OM) of Gram-negative bacteria poses a barrier to antibiotic entry due to its high impermeability. Thus, there is an urgent need to study the function and biogenesis of the OM. In *Enterobacteriales*, an order of bacteria with many pathogenic members, one of the components of the OM is enterobacterial common antigen (ECA). We have known of the presence of ECA on the cell surface of *Enterobacteriales* for many years, but its properties have only more recently begun to be unraveled. ECA is a carbohydrate antigen built of repeating units of three amino sugars, the structure of which is conserved throughout *Enterobacteriales*. There are three forms of ECA, two of which (ECA_{PG} and ECA_{LPS}) are located on the cell surface, while one (ECA_{CYC}) is located in the periplasm. Awareness of the importance of ECA has increased due to studies of its function that show it plays a vital role in bacterial physiology and interaction with the environment. Here, we review the discovery of ECA, the pathways for the biosynthesis of ECA, and the interactions of its various forms. In addition, we consider the role of ECA in the host immune response, as well as its potential roles in host-pathogen interaction. Furthermore, we explore recent work that offers insights into the cellular function of ECA. This review provides a glimpse of the biological significance of this enigmatic molecule.

KEYWORDS O-antigen, cross-reactivity, enterobacterial common antigen, outer membrane, surface antigens

Diverse environmental conditions on Earth (e.g., heat, pH, salinity, pressure, and osmotic activity) immensely affect the function of the cell, necessitating adaptation through structural modification. Gram-negative bacteria have an impermeable and strengthened outer membrane (OM) that allows them to withstand stress brought about by environmental factors, including other bacteria, antibiotics, and chemical stresses. The cell envelope structure of Gram-negative bacteria consists of the inner membrane, the periplasm containing the peptidoglycan cell wall, and the OM (1). The lipids of the OM form a barrier that is impermeable to large hydrophilic and hydrophobic molecules (2). Lipopolysaccharide (LPS) facilitates the formation of this barrier though (i) the high number of fatty acyl substituents per lipid molecule, which form a gel-like structure enhancing the rigidity of membrane (3, 4), (ii) strong lateral interaction between LPS molecules mediated by salt bridges with divalent cations (5), and (iii) modification of LPS structure in response to different environmental conditions (6). For example, in *Salmonella*, the PhoPQ two-component system causes antimicrobial peptide resistance after induction by divalent cation starvation by activating PagP (7). PagP facilitates the addition of palmitate chain to lipid A, altering the fluidity of the LPS molecules in the OM (7).

OM proteins (OMPs) are integral membrane proteins present in the membranes of Gram-negative bacteria, mitochondria, and chloroplasts. These proteins adopt a β -barrel architecture arranged in the membrane in anti-parallel patterns (8). Some of

Citation Rai AK, Mitchell AM. 2020.

Enterobacterial common antigen: synthesis and function of an enigmatic molecule. *mBio* 11:e01914-20. <https://doi.org/10.1128/mBio.01914-20>.

Editor Danielle A. Garsin, University of Texas Health Science Center at Houston

Copyright © 2020 Rai and Mitchell. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Angela M. Mitchell, amitchell@bio.tamu.edu.

Published 11 August 2020

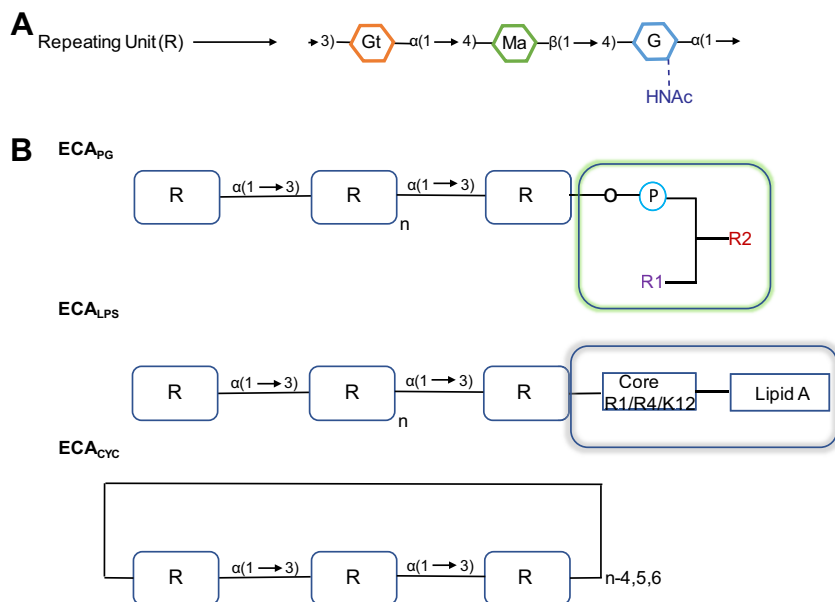


FIG 1 The structure of ECA. (A) The structure of the repeating unit (R) of ECA is made up of amino sugars (G, *N*-acetylglucosamine; Ma; *N*-acetyl-D-mannosaminuronic acid; Gt, 4-acetamido-4,6-dideoxy-D-galactose). (B) Structural differences between the three ECA forms. ECA_{PG} , phospholipid-linked ECA; ECA_{LPS} , lipopolysaccharide-linked ECA; ECA_{CYC} , cyclic form of ECA. In the ECA_{PG} structure, R1 (-CH₂OH group), and R2 (-CHOH group) indicate acyl chains. In the ECA_{LPS} structure, “core” represents the core polysaccharide of LPS, which is attached to lipid A, a hydrophobic lipid section that anchors LPS to the outer membrane. In the core region, the common tetrasaccharide structure is substituted for R1 (β -glucose) and R4 (β -galactose) compared to the K-12 core. *n*, a variable number of ECA repeating units. ECA_{CYC} generally consists of 4 to 6 repeating units (R) depending on the species. For example, in *E. coli*, 4 repeating units are present.

these proteins (porins) can form pores in the OM (2). These OMPs regulate the movement of small hydrophilic molecules across the outer membrane, such as nutrients, water, ions, and some small hydrophilic antibiotics (2). In fact, in *Escherichia coli*, β -lactam antibiotics, tetracyclines, chloramphenicol, and fluoroquinolones quickly diffuse through OmpF (9–12). Specific porins can also transport amphipathic substrates. For instance, transportation of long-chain fatty acid is facilitated by the lipid transporter FadL (13). Beyond its role controlling the entry of molecules into the cell, the OM plays a structural role, providing protection against mechanical and osmotic stresses (14, 15).

The Gram-negative OM is coated in highly variable molecules that can cause immune activation, known as antigens. Bacteria are divided into serotypes based on different antigen combinations (16). The three major types of antigens present on the cell surface are O (somatic), K (capsular), and H (flagellar) (17, 18). These antigens can play roles in motility (H-antigen), protection from a hostile environment (K-antigens and O-antigen), interaction with the environment (K-antigens and O-antigen), and increasing the ability of the OM to provide structural support to the cell (O-antigen) (15, 19–21). The outer leaflet of the OM is made mainly of lipopolysaccharide (LPS), which consists of lipid-A, the core polysaccharide, and O-antigen (1). O-antigen is a highly variable chain of carbohydrates and thus is serotype specific. K-antigens are the capsule, a coat on the surface of bacteria outside the cell envelope. They generally consist of high-molecular-weight polysaccharides with some exceptions (e.g., K-88 and K-99 of *E. coli*, which are protein antigens) (22–24). The H-antigen is a protein antigen based on flagellar structure (25). *Enterobacteriales* is a bacterial order that is defined in part by the presence of an antigen known as enterobacterial common antigen (ECA) (26). ECA, a carbohydrate antigen, is located in the outer leaflet of the OM and in the periplasm (27–30). Although *Enterobacteriales* express various antigens (e.g., K, O, and H) (31, 32), ECA is unique in that it is restricted to one order and in which it is invariant (Fig. 1A) allowing cross-reactivity among the members of *Enterobacteriales* (33).

Calvin M. Kunin and colleagues first discovered ECA in 1962 (33). The discovery of ECA was a result of studying strains of *E. coli* causing urinary tract infections and observing the reaction between rabbit antisera generated against the strains and 102 homologous and heterologous *E. coli* strains. The authors used a standard procedure (passive hemagglutination) to detect O-antigen found in the LPS of the *E. coli* (33). While carrying out these experiments, they realized there was a cross-reacting specificity between the antisera and many strains of *E. coli*. Although various antisera demonstrated differing reactivities, anti-*E. coli* O14 sera reacted with a remarkable range of strains: anti-O14 serum had antibodies recognizing an antigen common to various *E. coli* strains. However, this antigen was not the LPS-attached O-antigen that Kunin and colleagues had been investigating (33). Furthermore, this cross-reacting antigen was also observed in most other enteric bacteria (33, 34). The antigen was, therefore, named enterobacterial common antigen (ECA) (35).

After the discovery of ECA, research was conducted to ascertain the dissemination of the new antigen among species, eventually aided by a monoclonal ECA antibody that enhanced ECA detection (36). ECA is present in wild-type strains of *Enterobacteriales* and absent in both other Gram-negative bacteria and Gram-positive bacteria (Table 1). More studies need to be carried out on the unusual presence of enterobacterial common antigen in *Aeromonas hydrophila* 209A, as it is not present in the other strains belonging to the same species (37) and may be the result of horizontal gene transfer. Few exceptions to the ubiquitous expression of ECA in *Enterobacteriales* exist. These species, which appear to have lost ECA expression, are the endosymbiotic members of *Enterobacteriales*, which have a reduced genome size due to the loss of many genes rendered unnecessary by their obligate symbiotic life style (38, 39).

Antigens that are highly variable between strains of bacteria have served as the foundation for serological naming and grouping. For example, the Kauffmann-Perch scheme is used for *Proteus*, while the Kauffmann-White-Le Minor scheme is used for *Salmonella* (40–42). However, the importance of common antigens has often been overlooked. In recent times, the study of these antigens has increased given their potential significance in vaccine development, determination of phylogeny, and diagnosis. Furthermore, the invariance of common antigens suggests that they have important functions that do not allow for variability. ECA is a perfect example of an antigen that has undergone a recent resurgence of research despite its discovery many years ago. In this review, we explore the history of ECA, its interaction with the immune system, its isolation and biosynthesis, and finally its biological significance.

THE IMMUNOGENICITY OF ECA

Interactions of ECA with the immune response. ECA has a complex interaction with the immune response. Initial studies elucidated that, while the antigen occurred across *Enterobacteriales*, just a few sera had antibodies to ECA, for example, *E. coli* O14 (33, 43). Thus, all strains possessed antigenic ECA but very few possessed immunogenic ECA. The variance in immunogenicity of the strains studied could not be accounted for by differences in the amounts of ECA expressed (35, 44, 45). Therefore, something else must differentiate these types of ECAs. The elucidation of this difference came by separating ECA extracts with ethanol, in which LPS is not soluble, exposing a dissimilarity in the immunogenic types: an ethanol-insoluble immunogenic form and an ethanol-soluble nonimmunogenic form. The ethanol-insoluble form is not separable from LPS and signifies the immunogenic form of the enterobacterial common antigen (46). This form of ECA has ECA bound to the LPS core (ECA_{LPS}) (Fig. 1B). The ethanol-soluble form of ECA is not associated with LPS and, instead, consists of the ECA polysaccharide chain covalently linked to diacylglycerol through phosphodiester linkage (ECA_{PG}) (47). There is a third form of ECA, cyclic ECA (ECA_{CYC}); however, this molecule is found in the periplasm and is not exposed to the environment (48, 49).

Still, why some strains made immunogenic ECA_{LPS} while others did not remained unclear, in part because of the classification of the traditional ECA immunogenic strain, *E. coli* O14, as an O-antigen-positive strain (50). In fact, the strain is an irregular type of

TABLE 1 Distribution of ECA in Gram-negative bacteria

Family	ECA positive	ECA negative
Enterobacterales (194)		
<i>Budviciaceae</i>	<i>Leminorella</i>	
<i>Enterobacteriaceae</i>	<i>Pragia</i> <i>Atlantibacter</i> <i>Buttiauxella</i> <i>Cedecea</i> <i>Citrobacter</i> <i>Cronobacter</i> <i>Enterobacter</i> <i>Escherichia</i> <i>Gibbsiella</i> <i>Izhakiella</i> <i>Klebsiella</i> <i>Kluyvera</i> <i>Kosakonia</i> <i>Leclercia</i> <i>Lelliottia</i> <i>Limnobaculum</i> <i>Metakosakonia</i> <i>Pluralibacter</i> <i>Raoultella</i> <i>Salmonella</i> <i>Shigella</i> <i>Shimwellia</i>	"Candidatus"
<i>Erwiniaceae</i>	<i>Erwinia</i> <i>Mixta</i> <i>Pantoea</i> <i>Tatumella</i>	<i>Buchnera</i> , <i>Wigglesworthia</i>
<i>Hafniaceae</i>	<i>Edwardsiella</i> <i>Hafnia</i> <i>Obesumbacterium</i>	
<i>Morganellaceae</i>	<i>Arsenophonus</i>	"Candidatus Arsenophonus lipoptenae"
<i>Pectobacteriaceae</i>	<i>Morganella</i> <i>Photorhabdus</i> <i>Proteus</i> <i>Providencia</i> <i>Xenorhabdus</i> <i>Brenneria</i> <i>Dickeya</i> <i>Lonsdalea</i> <i>Pectobacterium</i> <i>Sodalis</i>	
<i>Yersiniaceae</i>	"Candidatus Fukatsuia" <i>Chania</i> <i>Rahnella</i> <i>Serratia</i> <i>Yersinia</i>	<i>Serratia symbiotica</i>
Unclassified	<i>Phytobacter</i> <i>Plesiomonas</i>	
<i>Vibrionales</i>		
<i>Vibrionaceae</i>	<i>Aeromonas hydrophila</i> 209A	<i>Vibrio</i>
Other Gram-negative bacteria		<i>Acidiferrobacter</i> <i>Actinobacillus</i> <i>Aeromonas</i> <i>Alcaligenes</i> <i>Bordetella</i> <i>Campylobacter</i> <i>Cardiobacterium</i> <i>Chromatiaceae</i> <i>Chromobacterium</i> <i>Colwellia</i>

(Continued on next page)

TABLE 1 (Continued)

Family	ECA positive	ECA negative
		<i>Eikenella</i>
		<i>Ferrimonas</i>
		<i>Flavobacterium</i>
		<i>Gardnerella</i>
		<i>Haemophilus</i>
		<i>Kingella</i>
		<i>Moraxella</i>
		<i>Moritella</i>
		<i>Shewanella</i>
		<i>Nitrobacteriaceae</i>
		<i>Pasteurella</i>
		<i>Pseudomonas</i>
		<i>Rhodospirillaceae</i>

the rough R4 strain disguised by the production of K7 capsular antigen (51). In combination with the production of immunogenic ECA_{LPS} by the R1, R4, and K-12 strains with rough LPS (43), this clarified that ECA_{LPS} is produced in significant amounts only by rough strains that do not make O-antigen. In nonimmunogenic strains, including O-antigen-producing smooth strains and rough strains with incomplete LPS cores or mutations in *waal*, the O-antigen ligase, ethanol-soluble ECA_{PG} is the predominant form of ECA on the cell surface (35). While some early studies suggested purified ECA_{PG} could induce an antibody response (52), this was only true in strains that also produced significant amounts of ECA_{LPS} (53), suggesting that the antibody production may have resulted from contaminating ECA_{LPS}.

With our current knowledge of immunology, it can now be appreciated how the differences in structure between ECA_{LPS} and ECA_{PG} would lead to differences in their immunogenicity. Antibody production is not efficiently stimulated without innate immune signaling (54). ECA_{LPS} possesses an intrinsic adjuvant to stimulate antibody production, as LPS is recognized by toll-like receptor 4 (TLR4), leading to the production of proinflammatory cytokines (55). Nevertheless, as a carbohydrate antigen, ECA_{LPS} mainly stimulates the production of IgM low-affinity, high-avidity antibodies (56). In contrast, the production of high-affinity IgG antibodies requires a protein antigen (57). As a proof of concept of the potential immunogenicity of ECA_{PG}, purified ECA_{PG} that contains proteins in addition to ECA can generate an immune response (52). Likewise, a conjugate of ECA and tetanus toxoid, a classic adjuvant, produces ECA antibodies mainly of the IgG isoform (56). Many of the initial studies on ECA immunogenicity were carried out with heat-killed bacteria (50). The difference in immunogenicity of ECA_{LPS} and ECA_{PG} is less in live bacteria (50), likely because of the many pathogen-associated molecular patterns (PAMPs) linked with an active infection and an increased production of proinflammatory cytokines (58).

Prevalence of ECA antibodies. Many early studies have reported a low titer of ECA antibodies present in human serum (33, 59, 60), with the caveat that these studies were conducted before the availability of an ECA knockout strain and so may report the combined titer of both ECA antibodies and antibodies to protein antigens shared among *Enterobacteriales* (e.g., OmpA). These antibodies have been found in both healthy donors and, at higher levels, in patients with chronic urinary tract infections (61). The titers of ECA antibodies present in the blood have been reported to increase with age (62). A maternal vaccination study reported that the cord blood of a child has lower amounts of ECA antibodies than maternal serum, showing some low level of maternal transfer (61). In other mammals such as cats, dogs, horses, pigs, and mice, ECA antibodies have been reported in blood sera with the exception of rabbits, where no ECA antibody is found. The likely reason for this is due to the high colonization of rabbits by Gram-positive bacteria and low prevalence of *E. coli* (60). Among several strains of mice, C57B1/6HA mice have ample ECA antibody titers after responding to ECA_{LPS} immunogens (63–65).

Estimates of ECA antibody prevalence in various types of infections have indicated variable titers of ECA antibodies (35). In a few diseases such as enteritis, bacteremia, and acute urinary tract infections, low titers of ECA antibodies were detected. However, higher ECA antibody titers were found in shigellosis (61, 66), peritonitis (53, 67), and chronic urinary tract infections (61, 68, 69). Surprisingly consistent levels of ECA antibodies were observed in a rabbit pyelonephritis model immunized by ECA_{LPS}, irrespective of the route of infection and diagnosis (70–72).

The presence of ECA antibodies has been detected in human serum after infection from pathogenic bacteria such as *E. coli*, *Yersinia enterocolitica* O3 strains (26, 73–75), and in humans suffering from arthritis associated with *Proteus mirabilis* strains (76). The ECA immunogenicity is mainly due to ECA_{LPS} (26), with exception to the Rc mutant R4/O28 of *P. mirabilis* in which ECA_{PG} provides immunogenicity. Therefore, the ECA can be used as a tool for serodetection in various infections caused by the members of the *Enterobacteriales* order (77).

Role of ECA antibodies in host-pathogen interactions. Several studies have attempted to determine whether ECA antibodies play a role, either protective or pathogenic, in various disease contexts. Under experimental conditions, an appreciable amount of ECA antibodies was found in mice immunized by *E. coli* O-antigen-negative strains and later challenged by pathogenic bacteria (63–65, 78), except in Swiss albino mice (65). However, protection from these immunizations was partial and temporary. The same partial protection was also observed in mice by passive immunization with serum from rabbits inoculated with *E. coli* O14 (63). Furthermore, a clinical study has demonstrated that passive immunization with a human monoclonal ECA antibody had no protective effect during sepsis caused by *Enterobacteriales* (79).

Bridge et al. (80) reported that, in a mouse model of salmonellosis, infection with the $\Delta wecA$ strain of *Salmonella enterica* serovar Typhimurium SL1344 via the oral or intraperitoneal route provided cross-protection against this infection by the production of IgG antibodies. In addition, Huang et al. (81) reported cross-protection against heterologous *Salmonella* strains in mice by downregulating the expression of O-antigen (*rmlB* [*rffB*]) and ECA (*rmlB*_{ECA} [*rffG*]) biosynthesis genes, allowing production of protein-recognizing antibodies. These studies suggest that, at least for *Salmonella*, ECA antibodies may not be protective and that ECA may “distract” the immune system from more efficacious targets. It should be mentioned that some studies have correlated the presence of antibodies to *Enterobacteriales*, including to ECA, with rheumatoid arthritis (82–84); however, the causal relationships leading to the antibody production remains unclear.

FORMS AND BIOSYNTHESIS OF ECA

Over the years, several researchers have developed methods for isolating ECA (Table 2). However, the chemical composition of the antigen was initially difficult to identify, due in part to the existence of ECA in three forms (26) (Fig. 1B). These forms are ECA linked to diacylglycerol through phosphodiester linkage (ECA_{PG}), LPS-linked ECA (ECA_{LPS}), and periplasmic cyclized ECA (ECA_{CYC}) (43, 47, 48). Observations made by Kunin, Neter, and Suzuki initially demonstrated that ECA occurred in two forms in the immunogenic *E. coli* O14. One of the forms was soluble in aqueous ethanol and was able to be separated from the LPS (ECA_{PG}), while the other did not dissolve in ethanol and could not be separated from LPS (ECA_{LPS}) (35, 37). ECA_{PG} is the predominant surface-exposed form of ECA, while ECA_{LPS}, the immunogenic form that allows antibody production, is predominantly found in rough mutants that do not make O-antigen (35). Later studies identified a third form of ECA, ECA_{CYC} (48), which is now thought to be present in all species that make ECA (48, 85–88). Initial studies done by Männel and Mayer in 1978 (89) reported that ECA consists of *N*-acetyl-D-glucosamine (GlcNAc) and *N*-acetyl-D-mannosaminuronic acid (ManNAcA). But in 1983, another component of ECA, 4-acetamido-4,6-dideoxy-D-galactose (Fuc4NAc), was identified in *Shigella* by Lugowski et al. (56), demonstrating that ECA consists of a trisaccharide repeating unit (Fig. 1A).

TABLE 2 Methods used for study of ECA

Methods used ^a	Representative species and strain(s)	Type of ECA	Representative reference(s)
Representative purification methods			
ECA_{LPS} Hot phenol-water extraction (water phase); dialysis; 90% ethanol precipitation (pellet); anion exchange chromatography	<i>Escherichia coli</i> O1, O14, O55; <i>Shigella flexneri</i>	ECA _{LPS}	52
LPS extraction and purification for analysis of ECA _{LPS} and other LPS forms	<i>Yersinia enterocolitica</i> O:3; <i>Shigella sonnei</i> phase II	ECA _{LPS} with LPS	141, 195
ECA_{PG} Lysis in boiling PBS (supernatant); 85% ethanol precipitation (supernatant)	<i>Salmonella enterica</i> serovar Typhimurium, <i>Salmonella choleraesuis</i> , <i>Salmonella enteritidis</i> , <i>S. flexneri</i> , <i>E. coli</i> O111, O55, O6, O75	ECA _{PG}	44, 72
Bacteria killed and dried with acetone; room temperature water extraction; picric acid precipitation (supernatant); acetone precipitation (pellet); Sephadex G200 column chromatography; preparative gel electrophoresis	<i>Salmonella typhosa</i> O901	ECA _{PG}	196
Hot phenol-water extraction (water phase); phenol-chloroform-petroleum ether extraction (phenol phase); ultracentrifugation (supernatant); anion exchange chromatography	<i>S. Montevideo</i> SH94, <i>S. Typhimurium</i> , <i>S. sonnei</i> phase I, <i>Plesiomonas shigelloides</i>	ECA _{PG}	81, 85, 89, 149, 197
ECA_{CYC} Sonication in EDTA and lysozyme; boiling water extraction; 85% ethanol precipitation (supernatant); acetone precipitation; column chromatography on silica gel and Sephadex LH-20	<i>S. sonnei</i>	ECA _{CYC}	48, 56, 140
Cold trichloroacetic acid extraction; Sephadex G-50 chromatography; anion exchange chromatography	<i>Yersinia pestis</i> EV	ECA _{CYC}	86
Sonication in MgSO ₄ ; ultracentrifugation (supernatant); 75% ethanol precipitation (supernatant); drying and resuspension in ddH ₂ O; desalting with ZipTip _{C18}	<i>E. coli</i> K-12	ECA _{CYC}	88, 111, 145
Hot phenol-water extraction (water phase); DNase, RNase, and protease treatment; ultracentrifugation (supernatant); size exclusion chromatography; Biogel P-100 chromatography	<i>E. coli</i> O157:H ⁻	ECA _{CYC} (no O-acetylation)	198
Common detection methods			
Passive HA; detection of antigen (whole cell, cell lysates, purified) by coating erythrocytes and assaying agglutination caused by reacting antibodies	<i>E. coli</i> O6, O75, OS:K27, K-12; <i>S. Typhimurium</i> TV149 (Ra); <i>S. Montevideo</i> SH94 (S); <i>S. sonnei</i> ; <i>P. shigelloides</i> ; <i>Plesiomonas rettgeri</i>	ECA _{LPS} ; ECA _{PG} ; ECA _{CYC} ; O-acetylation required for strong reactivity of serum with ECA	72, 85, 88, 89, 140, 197, 199
HA inhibition; detection by supernatant antigen prevention of agglutination of antigen-coated erythrocytes in the presence of antigen-specific antibody	<i>E. coli</i> O14, O6, O75, K-12; <i>S. Typhimurium</i> TV149 (Ra); <i>S. Montevideo</i> SH94 (S); <i>S. sonnei</i>	ECA _{LPS} ; ECA _{PG} ; ECA _{CYC} ; O-acetylation required for strong reactivity of serum with ECA	52, 72, 89, 111, 140, 199, 200
Immunodiffusion precipitation; identifies antigens after gel electrophoresis through precipitation caused by reaction with antibodies	<i>S. typhosa</i> O901; <i>S. Montevideo</i> SH94 (S); <i>S. sonnei</i>	ECA _{LPS} ; ECA _{PG}	89, 140, 196
ELISA; quantification of antigens based on their reaction with antibodies	<i>S. sonnei</i> ; <i>S. Montevideo</i> ; <i>P. shigelloides</i> ; <i>E. coli</i> OS:K27 ⁻ , K-12; <i>P. rettgeri</i>	ECA _{LPS} ; ECA _{PG}	85, 140, 197
Immunoblot; including SDS-PAGE or dot blot followed by immunoblot analysis	<i>S. Montevideo</i> SH94; <i>S. Typhimurium</i> ; <i>E. coli</i> R1, R4, OS:K27 ⁻ , K-12; <i>S. sonnei</i> ; <i>P. shigelloides</i> ; <i>P. rettgeri</i> ; <i>Y. enterocolitica</i> O:3	ECA _{LPS} ; ECA _{PG}	81, 141, 145, 197, 201
LC; including liquid-gas chromatography, HPLC, reverse-phase HPLC	<i>S. sonnei</i> ; <i>Y. pestis</i> ; <i>E. coli</i> K-12	ECA _{LPS} ; ECA _{PG} ; ECA _{CYC}	56, 86–88, 140

(Continued on next page)

TABLE 2 (Continued)

Methods used ^a	Representative species and strain(s)	Type of ECA	Representative reference(s)
NMR spectroscopy; including ¹ H, ¹³ C, and ³¹ P	<i>S. sonnei</i> ; <i>P. shigelloides</i> ; <i>Y. pestis</i> ; <i>S. Typhimurium</i> LT2; <i>E. coli</i> K-12, O157:H	ECA _{LPS} ; ECA _{PG} ; ECA _{CYC}	56, 85–87, 149, 195, 198
MS; including gas-LC-MS, gas chromatography-MS, matrix-assisted laser desorption-ionization time of flight	<i>P. shigelloides</i> ; <i>E. coli</i> K-12, O157:H ⁻ ; <i>S. sonnei</i>	ECA _{LPS} ; ECA _{PG} ; ECA _{CYC}	85, 88, 145, 195, 198

^addH₂O, double-distilled water; ELISA, enzyme-linked immunosorbent assay; HA, hemagglutination; HPLC, high-pressure liquid chromatography; LC, liquid chromatography; MS, mass spectroscopy; PBS, phosphate-buffered saline.

Biosynthesis of the ECA polymer. The synthesis of ECA is intricate, involving several phases (Fig. 2). The genes necessary for many steps in the synthesis of ECA are located within an operon known as the *wec* operon. The *wec* operon begins at position 85.4 centisomes on the *E. coli* K-12 chromosome (90), and the functions of each gene of the operon have been analyzed (90–98).

As for many other extracytoplasmic glycans (99), the assembly of the ECA trisaccharide repeat unit is carried out on an isoprenoid lipid carrier, undecaprenyl-phosphate (Und-P), a 55-carbon molecule made of isoprenoid units (93, 100–102). The assembly occurs on the inner side of the plasma membrane (103–107). The first step involves the formation of GlcNAc-pyrophosphoryl-undecaprenol, which is also known as lipid I^{ECA} (108). This step uses UDP-GlcNAc as a substrate to attach GlcNAc-1-phosphate to Und-P and is catalyzed by *WecA* (93, 101, 102). *In silico* predictions,

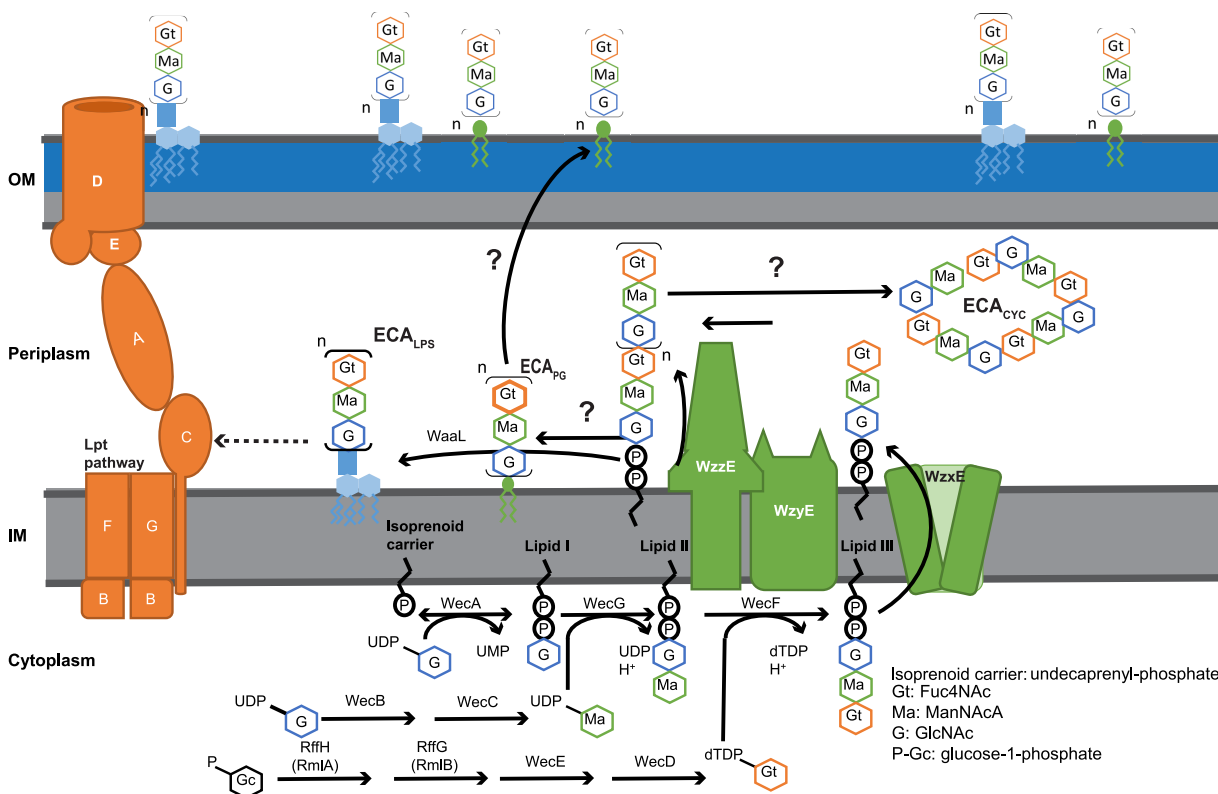


FIG 2 Schematic representation of ECA biogenesis in *E. coli*. ECA biogenesis begins with synthesis of amino sugars and their attachment to an isoprenoid carrier (Und-P). After a complete subunit is made by series of enzymes namely, *WecA*, *WecB*, *WecC*, *WecD*, *WecE*, *WecF*, *WecG*, *RmlA_{ECA}*, and *RmlB_{ECA}*, the precursor is flipped across the inner membrane by *Wzx*, and the subunits are polymerized on the isoprenoid carrier by *WzyE* with the chain length controlled by *WzzE*. Three forms of ECA are made from the polymerized subunits: ECA_{PG}, attached to diacylglycerol through phosphodiester linkage and surface exposed; ECA_{CYC}, which is periplasmic; and ECA_{LPS}, attached to LPS and surface exposed. This figure is adapted and modified from Mitchell et al. (145).

cysteine accessibility experiments, and fusion-protein expression experiments have demonstrated that WecA has 11 transmembrane segments, with the N terminus in the periplasm and the C terminus in the cytoplasm (109, 110). Furthermore, mutational studies have shown that several conserved aspartate residues in cytoplasmic loops 2 and 3 are important for WecA catalytic activity and divalent cation binding (110). Fluorescence microscopy for green fluorescent protein (GFP)-tagged WecA revealed that it is localized to punctate regions on the cell surface (110). The punctate localization of WecA suggests that ECA and/or O-antigen biosynthesis is localized to discrete cellular regions. The GlcNAc residue of ECA is nonstoichiometrically O-acetylated by WecH, an inner membrane O-acetylase (111).

WecB and WecC are responsible for synthesizing UDP-ManNAcA from UDP-GlcNAc (103, 112, 113). Specifically, WecB (UDP-N-acetylglucosamine 2-epimerase) reversibly epimerizes at carbon position 2 between UDP-GlcNAc and UDP-N-acetylmannosamine (112, 113). Campbell et al. (114) solved the structure of WecB at a 2.4-Å resolution. This homodimeric enzyme is comprised of two similar sandwich domains with the active site located in the deep cleft at the domain interface. Several basic residues in the active site may have a role in proton transfer at the C-2 position or stabilization of the oxy-carbonium ions in the transition state (114). Residues in the active site have been found to be important for allosteric regulation of WecB as well as for binding and catalysis (115). WecC oxidizes UDP-N-acetylmannosamine in the presence of NAD⁺ to form UDP-ManNAcA (116). The UDP-ManNAcA is the substrate to attach ManNAcA to the lipid I^{ECA}, a reaction carried out by WecG (101, 117). This process results in ManNAcA-GlcNAc-pyrophosphoryl-undecaprenol, also known as lipid II^{ECA}.

RmlA_{ECA} (RffH), RmlB_{ECA} (RffG), WecE, and WecD are responsible for synthesizing dTDP-Fuc4NAc from glucose-1-phosphate (103, 118, 119). The first reaction, carried out by RmlA_{ECA} (dTDP-glucose pyrophosphorylase 2), forms dTDP-glucose from glucose-1-phosphate, dTTP, and H⁺ (118, 120). Sivaraman et al. (120) solved the RmlA_{ECA} crystal structure in the presence of dTTP and Mg²⁺ ions at a 2.6-Å resolution. This enzyme is tetrameric with each monomer composed of an α/β fold with nucleotide-binding and sugar-binding domains. The active site was identified at the interface of two domains (120).

TDP-glucose acts as a substrate for second enzyme, RmlB_{ECA} (dTDP-glucose 4,6-dehydratase 2). RmlB_{ECA} converts dTDP-glucose to dTDP-4-keto-6-deoxy-D-glucose. This second reaction is a complex reaction that involves dehydrogenation, dehydration, and rereduction in the presence of cofactor NAD⁺ (121). Several active-site residues important for the function of RmlB_{ECA} have been identified based on similarity to UDP-galactose-4-epimerase and mutational analysis (122, 123).

The third reaction is catalyzed by WecE (dTDP-4-dehydro-6-deoxy-D-glucose transaminase), which converts dTDP-4-keto-6-deoxy-D-glucose to dTDP-4-amino-4,6-dideoxy- α -D-galactose (dTDP-Fuc4N) using glutamate as the amino donor (103, 124). A WecE crystal structure has been solved at a resolution of 2.24 Å (125). The structure indicates a homodimeric protein; however, a previous gel filtration experiment suggested a homotetrameric conformation (124). As is common for sugar aminotransferases, the WecE active site contains a conserved lysine that binds the catalytic cofactor, 5'-pyridoxal phosphate, an aspartate important for cofactor activation, and a conserved glutamine (125).

The last reaction is catalyzed by WecD (dTDP-fucosamine acetyltransferase) which uses acetyl coenzyme A (acetyl-CoA) as a cofactor to form dTDP-Fuc4NAc from dTDP-Fuc4N (103, 126). WecD has been crystallized at a resolution of 1.95 Å in its apo form and 1.66 Å in complex with acetyl-CoA (126). The structure shows a dimeric protein with each monomer adopting a GCN5-related N-acetyltransferase fold. WecF uses dTDP-Fuc4NAc to transfer Fuc4NAc to lipid II^{ECA}, forming Fuc4NAc-ManNAcA-GlcNAc-pyrophosphoryl-undecaprenol (lipid III^{ECA}) (103).

The synthesis of lipid III^{ECA} occurs on the inner leaflet of the cytoplasmic membrane facing the cytosol (103); however, polymerization of the trisaccharide repeat unit to form polysaccharide chains occurs on the outer leaflet of the cytoplasmic membrane

(127, 128). The flipping of lipid III^{ECA} to the periplasmic face of the membrane is mediated by a “flippase,” WzxE (127, 128). WzxE is a member of the polysaccharide-specific transporter family of proteins, which flip polysaccharides, including some O-antigens and capsular polysaccharides, across the inner membrane (IM) (128, 129). After translocation across the membrane, the ECA chain is polymerized by WzyE. The final chain length of the ECA polymer is determined by WzzE, the chain length regulator (91). These steps result in the formation of an ECA polymer attached to the isoprenoid carrier (Fig. 2).

Several studies have offered insights into the mechanism through which WzzE might control the chain length of ECA. Genetic evidence and cross-linking data support the notion that the flippase, polymerase, and chain length regulator work together as a complex (130, 131). Several structural studies have been performed with WzzE and other members of the class 1 polysaccharide copolymerase family (PCP-1) (132). A crystal structure of the periplasmic domain of WzzE, solved at 2.4 Å, revealed a bell-shaped homo-octameric structure (133); however, reports have suggested various oligomeric states for other PCP-1 family members depending on whether full-length protein was used and the method of analysis (134–137).

This structural inconsistency may be due to the lack of interactions with other complex members (i.e., WzyE). However, recent studies have again suggested octameric structure for both WzzE and WzzB (an *E. coli* O-antigen PCP-1) (136). The most recent structural data for PCP-1 proteins suggest a mechanism for PCP-1 chain length regulation where polymerization begins when the polymerase and PCP-1 form a complex with the growing polysaccharide chain wrapping either over the surface or through the cavity of the PCP-1 (137). The polymerization could then be terminated either when the PCP-1 and polymerase disassociate (a “stop-watch” mechanism) or when the polysaccharide-binding capacity of the PCP-1 is reached (a “molecular ruler” mechanism) (138–140).

Synthesis of the three forms of ECA. The three forms of ECA are made from the ECA polymer. ECA_{PG} is the dominant membrane-associated form of ECA and constitutes about 0.2% of the cellular dry weight of *E. coli* K-12 (26, 76, 140). This form is present in all *Enterobacteriales* (26). The polysaccharide chain is transferred from the isoprenoid carrier to an unidentified lipid to form ECA linked to diacylglycerol through phosphodiester linkage (ECA_{PG}) (47). In this molecule, ECA is the head group of the phospholipid (47). The newly synthesized ECA_{PG} is then translocated to the outer membrane (27, 29). The genes and mechanisms involved in the synthesis and translocation of ECA_{PG} remain unknown (87). ECA_{LPS} is synthesized by transferring the linear ECA polysaccharide chain to the core oligosaccharide of LPS (26, 46). This step is catalyzed by WaaL, which is also responsible for attaching O-antigen to the core of LPS (43, 51). However, data suggest that the method for attaching ECA to LPS in *Yersinia enterocolitica* may be different, allowing ECA and O-antigen to coexist (141). The last form of ECA, ECA_{CYC}, is a cyclic molecule consisting of polymerized ECA trisaccharide repeat units, and it is water soluble (26, 48). The ECA_{CYC} is localized in the periplasm (88). This polymer has a variable number of repeat units (4–6) depending on the species (91), and the chain length regulator, WzzE, is necessary for its synthesis (48, 87, 88). A cyclase has not been identified. In-depth structural analysis by crystallography, nuclear magnetic resonance (NMR), and molecular dynamics have suggested that ECA_{CYC} can exist in two three-dimensional conformations (142–144). In contrast to ECA_{CYC}, ECA_{PG} and ECA_{LPS} consist of 1 to 14 repeat units, with a modal value of 5 to 7 units in *E. coli* K-12 (91). In addition, different modal chain lengths have been observed depending on the growth temperature (145).

Interactions with other biosynthetic pathways. The use of isoprenoid carriers, such as Und-P, for the synthesis of extracytoplasmic glycans is highly conserved across the domains of life (99, 146, 147). Furthermore, these carriers are often utilized for the synthesis of multiple glycans in the same species. For instance, in *E. coli*, Und-P is used for the production of ECA, O-antigen, peptidoglycan, and the colanic acid capsule (43,

148–151). Thus, Und-P is a universal lipid carrier required for the synthesis of numerous glycan polymers (152), and this can lead to complex interactions between biosynthetic pathways.

Disruption of one Und-P pathway may lead to indirect consequences on other glycans by altering the amount of Und-P and other precursors available for their synthesis. For example, obstructing the O-antigen pathway in *E. coli* compromises peptidoglycan biosynthesis by sequestering Und-P (153). In relation to ECA, it was first observed that disruption of later steps in ECA biosynthesis that lead to the accumulation of lipid II^{ECA} causes detergent sensitivity (154) and bile salt sensitivity (155). It was then found that these mutations also lead to the activation of extracytoplasmic stress responses, including Cpx, σ^E , and Rcs (156–158). Interestingly, in *E. coli* and *Salmonella enterica*, these stress responses are only activated with mutations that cause lipid II^{ECA} accumulation (147, 156), but in *Serratia marcescens*, Rcs activation has been reported even in strains with mutations early in ECA biosynthesis (158). The link of detergent sensitivity and stress response activation to defects in peptidoglycan biosynthesis was conclusively established by the observation that mutations leading to the accumulation of lipid II^{ECA} cause changes in cell shape due to sequestering of Und-P (159). While the accumulation of lipid II^{ECA} is deleterious to the cell, in *E. coli*, the accumulation of lipid III^{ECA} has been shown to be lethal (88, 128). This has been observed with both loss of WzyE, the ECA polymerase, and loss of the capacity to flip lipid III^{ECA} across the inner membrane (see below) (88, 128). Avoiding lipid III^{ECA} accumulation by disrupting an earlier step in ECA biosynthesis prevents this lethality (88).

The ECA and O-antigen biosynthesis pathways are homologous in *Enterobacteriales*. All members of *Enterobacteriales* utilize the *wec* locus for the biosynthesis of ECA (102, 160). However, many *Enterobacteriales* with GlcNAc as their first O-antigen residue (e.g., *Salmonella enterica* serovar Minnesota [161], *Salmonella enterica* serovar Montevideo [162], and *E. coli* [43]) also utilize *wecA* (*rfe*), the first gene in ECA biosynthesis, for the production of O-antigen chains (163). Therefore, disruptions of *wecA* result in loss of both O-antigen and ECA biosynthesis (43, 164). In addition, the functions of RmlA_{ECA} and RmlB_{ECA}, which function in the synthesis of dTDP-Fuc4NAc for ECA biosynthesis, are at least partially redundant with RmlA and RmlB, respectively, two enzymes involved in the synthesis of dTDP-L-rhamnose for O-antigen biosynthesis (118).

The absence of all Wzx family flippases (Wzx_{O16}, WzxC, and WzxE) in *E. coli* K-12 leads to a lethal accumulation of lipid III^{ECA}, which can be prevented by the expression of any of the flippases or by prevention of ECA synthesis at an earlier step (88, 128). However, with wild-type expression of *wzx*_{O16} and *wzx*C, deletion of *wzx*E is not lethal, and approximately wild-type levels of ECA_{CYC} are produced (88). These data can be explained by the complex specificity of Wzx family flippases, which has recently been thoroughly reviewed (165). Recent work from Liu et al. (166) suggests “structure-specific triggering,” where flipping is triggered by recognition of specific structural element(s) and other “incorrect” O-antigens may trigger flipping at low frequency when the “correct” substrate is absent (166).

Under normal conditions, the expression of Wzx flippases is low (167), and these flippases show specificity to their canonical substrates (166, 168–172). With various Wzx proteins, the specificity that triggers flipping has been found to be the first sugar residue attached to the Und-PP carrier (168, 169), the presence of terminal side branch residues (170, 171), or the identity or linkage of the terminal residue of the oligosaccharide (166, 171, 172). In the absence of their canonical substrate, the flippases can transport other Und-PP-linked oligosaccharides with various degrees of efficiency depending on their structural similarity, and this transport can be increased by over-expression (166, 170, 171). Thus, in *E. coli* K-12, which is O-antigen negative but would produce O-antigen with an initial GlcNAc and which does not produce colanic acid unless stressed, the O-antigen and colanic acid Wzx proteins can be utilized with enough efficiency by ECA to prevent a lethal accumulation of lipid III^{ECA} and to produce a measurable accumulation of ECA (88), although the process may be inefficient.

TABLE 3 Biological significance of ECA in *Enterobacteriales*

Function	Type of ECA	Associated gene(s)	Reference(s)
Inhibition of P22 lysis in <i>Salmonella enterica</i>	Complete biosynthesis disruption, possible peptidoglycan stress	<i>wecB</i> , <i>wecC</i> , <i>wecD</i> , <i>wecE</i> , <i>wecG</i> , and <i>wzxE</i>	176
Virulence in <i>S. Typhimurium</i>	Loss of all forms of ECA	<i>wecA</i> , <i>wecD</i>	147, 162
Resistance to toxic molecules (e.g., bile salt, acetic acid, serum, and antibiotics)	Complete loss of ECA, loss O-antigen in some species	<i>wecA</i>	150, 160, 178, 181, 182
Resistance to gentamycin	Accumulation of lipid II ^{ECA} ; peptidoglycan stress	<i>wecE</i>	179
Resistance to nalidixic and amikacin	Accumulation of lipid III ^{ECA} ; peptidoglycan stress	<i>wzxE</i>	180
Maintenance of OM permeability barrier and resistance to detergent and bile salt	ECA _{CYC}		111, 145
Proposed regulation of Ca ²⁺ ions in the cell	ECA _{PG}		52
Maintenance of cell membrane integrity in <i>S. marcescens</i>	Loss of all forms of ECA		183

Finally, WaaL, the O-antigen ligase, is responsible for attaching both O-antigen and ECA to LPS (43). Thus, the level of ECA_{LPS} greatly depends on the presence or absence of O-antigen, with very little ECA_{LPS} produced in O-antigen plus strains in *E. coli* (35). However, *Yersinia enterocolitica* and *Proteus mirabilis* produce significant amounts of ECA_{LPS} even in the presence of O-antigen (76, 105). Thus, the interactions between ECA biosynthesis, O-antigen, and peptidoglycan biosynthesis pathways are highly complex. One consequence of these complex interactions is that it confounds the interpretation of high-throughput genetic screens identifying functions of genes in ECA biosynthesis (173–176).

BIOLOGICAL SIGNIFICANCE OF ECA

The order *Enterobacteriales* contains many pathogens important to living organisms, including human beings. Early studies showed that when bacteria are subcultured for prolonged periods under laboratory conditions, the capability to synthesize O-chains diminishes, but there are no effects on the stability of ECA (37). Thus, ECA must have a vital role in *Enterobacteriales* (Table 3). However, efforts to clarify the specific biological role of ECA for the enterobacterial cell have failed, partially because of the many interactions between the O-antigen, peptidoglycan (PG), and ECA biosynthesis pathways (43, 144, 156–159). Due to this, very few unambiguously assigned functions have been ascribed to ECA.

There has been no difference observed in both the autolysis and the viability in cells having ECA and their counterparts lacking the antigen (177). Barua et al. (178) have shown in the Shiga-toxin-producing *E. coli* strain O157:H7 that mutants in *wecA* and *wecB* resulting in the loss of ECA and O-antigen or ECA alone, respectively, are sensitive to acetic acid. In addition, there is an increased sensitivity to some antibiotics in mutants lacking ECA, especially aminoglycosides, for example, kanamycin and gentamicin (177). A similar observation was made in *E. coli* where a *wecE* mutant was found to be sensitive to gentamicin (179). In addition, Girgis et al. (180) observed that a *wzxE* mutant has no phenotype in neutral agar media but, in the presence of nalidixic acid and amikacin, the mutant is sensitive compared to the wild type. A large-scale chemical genetics screen suggested that a *wecA* mutant of *E. coli* K-12 lacking all ECAs was sensitive to azidothymidine, CHIR-90, minocycline, procaine, puromycin, triclosan, and trimethoprim and resistant to fusidic acid, isoniazid, amdinocillin, vancomycin, and polymyxin (181). A comparison between the ECA_{PG} and the lipopolysaccharide of *Salmonella* Montevideo showed that the ECA_{PG} has a higher Ca²⁺-to-Mg²⁺ ratio than lipopolysaccharide, hence the suggestion that enterobacterial common antigen is important for the supply of calcium ions in the cell (52). Random-transposon mutagenesis experiments performed in *Salmonella enterica* revealed that disruption of six of the ECA operon genes (*wecB*, *wecC*, *wecD*, *wecE*, *wecG*, and *wzxE*) led to increased speed of lysis by bacteriophage P22 (176). As no effect was observed for disruption of *wecA*, it is possible that this effect is due to the peptidoglycan stress caused by these mutations.

One of the critical roles of ECA is the pathogenicity of *Enterobacteriales*, which has been found in some studies. For instance, *Salmonella enterica* lacking ECA (with mutations in either *wecD* or *wecA*) becomes less virulent and more sensitive to bile salts (147), although it does not use the *wecA* gene for O-antigen biosynthesis, as do many *Enterobacteriales*. The phenotype of the *wecD* mutant was more severe than that for the *wecA* mutation, likely due to an accumulation of a Und-P-linked ECA precursor disrupting peptidoglycan synthesis. Gilbreath et al. (182) further validated this result. *In vitro* they found that a *wecA* null mutant of *S. Typhimurium* is deficient in ECA production but fully competent for O-antigen production and lacks stress response activation caused by peptidoglycan biosynthesis disruption. This mutant was highly attenuated in mice, causing a persistent low-level infection that did not kill the mice (182).

Interestingly, mutants defective in ECA biosynthesis trigger Rcs stress response activation in *Serratia marcescens* regardless of whether peptidoglycan biosynthesis is disrupted, suggesting that ECA may play an especially important role in envelope integrity for this species (150). A role in envelope integrity is also suggested by the overproduction of outer membrane vesicles (OMV) in the absence of ECA in *Serratia marcescens*, suggesting an instability in the OM (183). In contrast, a screen in *E. coli* K-12 revealed differences in OMV production in ECA biosynthesis mutants, but these phenotypes varied greatly depending on which gene was mutated, suggesting that the results may be indirect (184). Phan et al. (174) found that seven genes of the ECA operon are essential for serum resistance in *E. coli*; however, these effects may have been the result of the loss of O-antigen and/or isoprenoid carrier effects.

Recently, we determined that one of the forms of ECA, ECA_{CYC}, plays a significant role in the OM permeability barrier (145). We found that loss of ECA_{CYC} disrupts the OM permeability barrier, causing detergent and bile salt sensitivity. Furthermore, we determined that ECA_{CYC} genetically interacts with a protein of unknown function, YhdP, to carry out this activity. When *yhdP* is deleted, ECA_{CYC} takes on aberrant activity that damages the OM, despite greatly lowered ECA_{CYC} levels. Careful screening of different ECA mutants and screening for hallmarks of peptidoglycan stress allowed us to eliminate peptidoglycan stress as a cause of these phenotypes (145).

ECA has been considered as a vaccination against infections stemming from enterobacterial strains due to its prevalence within the order. In a mouse model, salmonellosis leads to the development of ECA antibodies. However, no insight into the role of ECA antibodies in protection from infection was provided (185). Results regarding the protective nature of ECA antibodies have been mixed (63–65, 78, 79, 81). Further investigation into the protective nature of ECA antibodies against *Enterobacteriales* species is warranted, as ECA antibodies have the potential to protect against all enterobacterial pathogens. In an ECA vaccine candidate, it would likely be important that the O-acetylation of ECA be maintained. O-Acetylation of surface-exposed polysaccharide has been shown to be important for the generation of protective antibodies for many pathogens (186–191). Kajimura et al. (111) have reported that the absence of O-acetylation in ECA_{CYC} decreases the immunoreactivity of this ECA form toward a rabbit-generated antibody. A recent study has determined that a partially O-acetylated polysaccharide may be highly advantageous for vaccine use due to epitope exposure and hydrophobicity profiles (192). In this aspect, ECA may be ideal given its nonstoichiometric acetylation of GlcNAc (111).

CONCLUDING REMARKS

Since the discovery of ECA in 1962, the work of many investigators has elucidated considerable information about the structure and biogenesis of ECA, and yet our understanding of this fascinating molecule remains incomplete. Whereas many carbohydrate antigens on the bacterial cell surface are highly variable allowing for escape from immune surveillance, ECA remains invariant despite its presence in many pathogenic species. This suggests that the function of ECA must be incredibly important for the cell. Yet, this function is largely unknown. In the past, investigations of the function of ECA have been hampered by the complex genetic and biosynthetic interactions

between ECA and other cytoplasmic glycans (i.e., peptidoglycan and O-antigen). Now that these interactions have been characterized, more in-depth studies of ECA functions will be possible.

It is quite likely that the functions of ECA will be found to vary between the types of ECA and to occur on the level of cellular function as well as interaction with the environment. For instance, it has become clear that ECA_{CYC} and ECA_{PG} both play roles in maintaining the OM permeability barrier (145); however, given that antibiotic and detergent susceptibility differs between the loss of these two molecules and the different cellular location of the ECA forms, it is likely that the function of each is distinct. ECA_{LPS}, as of yet, has had no cellular function described. While it is clear that ECA is important for pathogenesis, at least in *Salmonella*, it has not yet been determined whether this is due to an alteration of *Salmonella* cellular function or an alteration of *Salmonella*'s interaction with the host.

Nevertheless, from the earliest studies of ECA it has been shown that ECA interacts with the host immune system, leading to the production of broadly cross-reactive antibodies. Yet, it is not known whether these ubiquitous antibodies play a role in the pathogenesis or protection from enterobacterial pathogens. Further evidence of the importance of ECA interactions with the environment can be gleaned from the regulation of ECA expression or chain length by temperature (141, 145, 193). Future investigations into the functions of ECA will lend important insights into the cellular function and host-pathogen interactions of an important group of bacteria.

ACKNOWLEDGMENTS

We thank members of the Mitchell Lab for productive discussions.

This work was supported by startup funds from Texas A&M University.

REFERENCES

- Silhavy TJ, Kahne D, Walker S. 2010. The bacterial cell envelope. *Cold Spring Harb Perspect Biol* 2:a000414. <https://doi.org/10.1101/cshperspect.a000414>.
- Nikaido H. 2003. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev* 67:593–656. <https://doi.org/10.1128/MMBR.67.4.593-656.2003>.
- Labischinski H, Barnickel G, Bradaczek H, Naumann D, Rietschel ET, Giesbrecht P. 1985. High state of order of isolated bacterial lipopolysaccharide and its possible contribution to the permeation barrier property of the outer membrane. *J Bacteriol* 162:9–20. <https://doi.org/10.1128/JB.162.1.9-20.1985>.
- Plesiat P, Nikaido H. 1992. Outer membranes of Gram-negative bacteria are permeable to steroid probes. *Mol Microbiol* 6:1323–1333. <https://doi.org/10.1111/j.1365-2958.1992.tb00853.x>.
- Papahadjopoulos D, Portis A, Pangborn W. 1978. Calcium-induced lipid phase transitions and membrane fusion. *Ann N Y Acad Sci* 308:50–66. <https://doi.org/10.1111/j.1749-6632.1978.tb22013.x>.
- Simpson BW, Trent MS. 2019. Pushing the envelope: LPS modifications and their consequences. *Nat Rev Microbiol* 17:403–416. <https://doi.org/10.1038/s41579-019-0201-x>.
- Guo L, Lim KB, Poduje CM, Daniel M, Gunn JS, Hackett M, Miller SI. 1998. Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides. *Cell* 95:189–198. [https://doi.org/10.1016/S0092-8674\(00\)81750-X](https://doi.org/10.1016/S0092-8674(00)81750-X).
- Fairman JW, Noinaj N, Buchanan SK. 2011. The structural biology of β -barrel membrane proteins: a summary of recent reports. *Curr Opin Struct Biol* 21:523–531. <https://doi.org/10.1016/j.sbi.2011.05.005>.
- Harder KJ, Nikaido H, Matsushashi M. 1981. Mutants of *Escherichia coli* that are resistant to certain beta-lactam compounds lack the *ompF* porin. *Antimicrob Agents Chemother* 20:549–552. <https://doi.org/10.1128/AAC.20.4.549>.
- Hooper DC, Wolfson JS, Souza KS, Tung C, McHugh GL, Swartz MN. 1986. Genetic and biochemical characterization of norfloxacin resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 29:639–644. <https://doi.org/10.1128/AAC.29.4.639>.
- Pugsley AP, Schnaitman CA. 1978. Outer membrane proteins of *Escherichia coli* VII. Evidence that bacteriophage-directed protein 2 functions as a pore. *J Bacteriol* 133:1181–1189. <https://doi.org/10.1128/JB.133.3.1181-1189.1978>.
- Reeve EC, Doherty P. 1968. Linkage relationships of two genes causing partial resistance to chloramphenicol in *Escherichia coli*. *J Bacteriol* 96:1450–1451. <https://doi.org/10.1128/JB.96.4.1450-1451.1968>.
- Ginsburgh CL, Black PN, Nunn WD. 1984. Transport of long chain fatty acids in *Escherichia coli*. Identification of a membrane protein associated with the *fadL* gene. *J Biol Chem* 259:8437–8443.
- Berry J, Rajaure M, Pang T, Young R. 2012. The spanin complex is essential for lambda lysis. *J Bacteriol* 194:5667–5674. <https://doi.org/10.1128/JB.01245-12>.
- Rojas ER, Billings G, Odermatt PD, Auer GK, Zhu L, Miguel A, Chang F, Weibel DB, Theriot JA, Huang KC. 2018. The outer membrane is an essential load-bearing element in Gram-negative bacteria. *Nature* 559:617–621. <https://doi.org/10.1038/s41586-018-0344-3>.
- van Wuijkhuijse AL, van Baar BL. 2008. Recent advances in real-time mass spectrometry detection of bacteria, p 929–954. *Principles of bacterial detection: biosensors, recognition receptors and microsystems*. Springer, New York, NY.
- Whitfield C, Roberts IS. 1999. Structure, assembly and regulation of expression of capsules in *Escherichia coli*. *Mol Microbiol* 31:1307–1319. <https://doi.org/10.1046/j.1365-2958.1999.01276.x>.
- Robins-Browne RM, Hartland EL. 2002. *Escherichia coli* as a cause of diarrhea. *J Gastroenterol Hepatol* 17:467–475. <https://doi.org/10.1046/j.1440-1746.2002.02769.x>.
- Lerouge I, Vanderleyden J. 2002. O-antigen structural variation: mechanisms and possible roles in animal/plant–microbe interactions. *FEMS Microbiol Rev* 26:17–47. <https://doi.org/10.1111/j.1574-6976.2002.tb00597.x>.
- Wang L, Rothmund D, Curd H, Reeves PR. 2003. Species-wide variation in the *Escherichia coli* flagellin (H-antigen) gene. *J Bacteriol* 185:2936–2943. <https://doi.org/10.1128/JB.185.9.2936-2943.2003>.
- Williams P, Lambert PA, Brown MR, Jones RJ. 1983. The role of the O and K antigens in determining the resistance of *Klebsiella aerogenes* to serum killing and phagocytosis. *Microbiology* 129:2181–2191. <https://doi.org/10.1099/00221287-129-7-2181>.
- Ørskov I, Ørskov F, Sojka W, Leach J. 2009. Simultaneous occurrence of

- E. coli* B and L antigens in strains from diseased swine: influence of cultivation temperature two new *E. coli* K antigens: K 87 and K 88. *Acta Pathol Microbiol Scand* 53:404–422. <https://doi.org/10.1111/j.1699-0463.1961.tb00424.x>.
23. Smith HW, Linggood MA. 1971. Observations on the pathogenic properties of the K88, Hly and Ent plasmids of *Escherichia coli* with particular reference to porcine diarrhoea. *J Med Microbiol* 4:467–485. <https://doi.org/10.1099/00222615-4-4-467>.
 24. Ørskov I, Ørskov F, Smith HW, Sojka W. 2009. The establishment of K99, a thermolabile, transmissible *Escherichia coli* K antigen, previously called “Kco”, possessed by calf and lamb enteropathogenic strains. *Acta Pathol Microbiol Scand B Microbiol* 83B:31–36. <https://doi.org/10.1111/j.1699-0463.1975.tb00066.x>.
 25. Ørskov F, Ørskov I. 1978. Chapter I. Serotyping of *Enterobacteriaceae*, with special emphasis on K antigen determination, p 1–77. *Methods in microbiology*, vol 11. Elsevier, Amsterdam, Netherlands.
 26. Kuhn H-M, Meier-Dieter U, Mayer H. 1988. ECA, the enterobacterial common antigen. *FEMS Microbiol Rev* 4:195–222. <https://doi.org/10.1111/j.1574-6968.1988.tb02743.x>.
 27. Rinno J, Golecki J, Mayer H. 1980. Localization of enterobacterial common antigen: immunogenic and nonimmunogenic enterobacterial common antigen-containing *Escherichia coli*. *J Bacteriol* 141:814–821. <https://doi.org/10.1128/JB.141.2.814-821.1980>.
 28. Acker G, Schmidt G, Mayer H. 1982. Accessibility of enterobacterial common antigen to antibodies in encapsulated and non-encapsulated S and R forms of *Escherichia coli*. *Microbiology* 128:1577–1583. <https://doi.org/10.1099/00221287-128-7-1577>.
 29. Acker G, Bitter-Suermann D, Meier-Dieter U, Peters H, Mayer H. 1986. Immunocytochemical localization of enterobacterial common antigen in *Escherichia coli* and *Yersinia enterocolitica* cells. *J Bacteriol* 168:348–356. <https://doi.org/10.1128/JB.168.1.348-356.1986>.
 30. Acker G. 1988. Immunoelectron microscopy of surface antigens (polysaccharides) of Gram-negative bacteria using pre- and post-embedding techniques, p 147–174. *Methods in microbiology*, vol 20. Academic Press, San Diego, CA.
 31. Jann K, Westphal O. 1975. Microbial polysaccharides, p 1–125. *The antigens*. Elsevier, Amsterdam, Netherlands.
 32. Weibull C, Högfeldt E, Sillén LG, Kinell P-O. 1950. Investigations on bacterial flagella. *Acta Chem Scand* 4:268–276. <https://doi.org/10.3891/acta.chem.scand.04-0268>.
 33. Kunin CM, Beard MV, Halmagyi NE. 1962. Evidence for a common hapten associated with endotoxin fractions of *E. coli* and other *Enterobacteriaceae*. *Proc Soc Exp Biol Med* 111:160–166. <https://doi.org/10.3181/00379727-111-27734>.
 34. Le Minor L, Chalon A, Veron M. 1972. Recherches sur la présence de l’antigène commun des “*Enterobacteriaceae*” (antigène Kunin) chez les “*Yersinia*” “*Levinea*” “*Aeromonas*” et “*Vibrio*”. *Ann Inst Pasteur (Paris)* 123:761–774.
 35. Mäkelä P, Mayer H. 1976. Enterobacterial common antigen. *Bacteriol Rev* 40:591–632. <https://doi.org/10.1128/MMBR.40.3.591-632.1976>.
 36. Böttger E, Jürs M, Barrett T, Wachsmuth K, Metzger S, Bitter-Suermann D. 1987. Qualitative and quantitative determination of enterobacterial common antigen (ECA) with monoclonal antibodies: expression of ECA by two *Actinobacillus* species. *J Clin Microbiol* 25:377–382. <https://doi.org/10.1128/JCM.25.2.377-382.1987>.
 37. Mayer H, Schmidt G. 1979. Chemistry and biology of the enterobacterial common antigen (ECA), p 99–153. *Current topics in microbiology and immunology*. Springer, New York, NY.
 38. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, von Mering C. 2019. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 47:D607–D613. <https://doi.org/10.1093/nar/gky1131>.
 39. McCutcheon JP, Boyd BM, Dale C. 2019. The life of an insect endosymbiont from the cradle to the grave. *Curr Biol* 29:R485–R495. <https://doi.org/10.1016/j.cub.2019.03.032>.
 40. Kauffmann F. 1966. *The bacteriology of the Enterobacteriaceae*, p 333–360. The Williams & Wilkins Co., Baltimore, MD.
 41. Perch B. 2009. On the serology of the *Proteus* group. *Acta Pathol Mic Sc* 25:703–714. <https://doi.org/10.1111/j.1699-0463.1948.tb00712.x>.
 42. Grimont PA, Weill FX. 2007. *Antigenic formulae of the Salmonella serovars*, 9th ed. WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France.
 43. Schmidt G, Männel D, Mayer H, Whang H, Neter E. 1976. Role of a lipopolysaccharide gene for immunogenicity of the enterobacterial common antigen. *J Bacteriol* 126:579–586. <https://doi.org/10.1128/JB.126.2.579-586.1976>.
 44. Suzuki T, Gorzynski E, Neter E. 1964. Separation by ethanol of common and somatic antigens of *Enterobacteriaceae*. *J Bacteriol* 88:1240–1243. <https://doi.org/10.1128/JB.88.5.1240-1243.1964>.
 45. Gorzynski E, Whang H, Suzuki T, Neter E. 1963. Differences in antibody response of rabbit to enterobacterial antigen after intravenous and subcutaneous injection with adjuvant. *Proc Soc Exp Biol Med* 114:700–704. <https://doi.org/10.3181/00379727-114-28775>.
 46. Kiss P, Rinno J, Schmidt G, Mayer H. 1978. Structural studies on the immunogenic form of the enterobacterial common antigen. *Eur J Biochem* 88:211–218. <https://doi.org/10.1111/j.1432-1033.1978.tb12440.x>.
 47. Kuhn HM, Neter E, Mayer H. 1983. Modification of the lipid moiety of the enterobacterial common antigen by the “*Pseudomonas* factor”. *Infect Immun* 40:696–700. <https://doi.org/10.1128/IAI.40.2.696-700.1983>.
 48. Dell A, Oates J, Lugowski C, Romanowska E, Kenne L, Lindberg B. 1984. The enterobacterial common-antigen, a cyclic polysaccharide. *Carbohydr Res* 133:95–104. [https://doi.org/10.1016/0008-6215\(84\)85186-1](https://doi.org/10.1016/0008-6215(84)85186-1).
 49. Dell A, Panico M. 1986. *In* Gaskel S (ed), *Mass spectrometry in biomedical research*. Wiley, New York, NY.
 50. Whang H, Mayer H, Schmidt G, Neter E. 1972. Immunogenicity of the common enterobacterial antigen produced by smooth and rough strains. *Infect Immun* 6:533–539. <https://doi.org/10.1128/IAI.6.4.533-539.1972>.
 51. Schmidt G, Jann B, Jann K. 1974. Genetic and immunochemical studies on *Escherichia coli* O14:K7:H. *Eur J Biochem* 42:303–309. <https://doi.org/10.1111/j.1432-1033.1974.tb03340.x>.
 52. Kuhn HM, Adamus G, Romanowska E, Mayer H. 1981. Effect of proteins on the immunogenicity of enterobacterial common antigen. *Infect Immun* 34:373–377. <https://doi.org/10.1128/IAI.34.2.373-377.1981>.
 53. Neter E, Whang HY, Mayer H. 1973. Immunogenicity and antigenicity of endotoxic lipopolysaccharides: reversible effects of temperature on immunogenicity. *J Infect Dis* 128:S56–S60. https://doi.org/10.1093/infdis/128.Supplement_1.S56.
 54. Chaplin DD. 2010. Overview of the immune response. *J Allergy Clin Immunol* 125:S3–S23. <https://doi.org/10.1016/j.jaci.2009.12.980>.
 55. Park BS, Lee JO. 2013. Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp Mol Med* 45:e66. <https://doi.org/10.1038/emmm.2013.97>.
 56. Lugowski C, Kułakowska M, Romanowska E. 1983. Enterobacterial common antigen-tetanus toxoid conjugate as immunogen. *Infect Immun* 42:1086–1091. <https://doi.org/10.1128/IAI.42.3.1086-1091.1983>.
 57. Schroeder HW, Jr, Cavacini L. 2010. Structure and function of immunoglobulins. *J Allergy Clin Immunol* 125:S41–S52. <https://doi.org/10.1016/j.jaci.2009.09.046>.
 58. Mogensen TH. 2009. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin Microbiol Rev* 22:240–273. <https://doi.org/10.1128/CMR.00046-08>.
 59. Kunin CM. 1963. Separation, characterization, and biological significance of a common antigen in *Enterobacteriaceae*. *J Exp Med* 118:565–586. <https://doi.org/10.1084/jem.118.4.565>.
 60. Kunin CM, Beard MV. 1963. Serological studies of O antigens of *Escherichia coli* by means of the hemagglutination test. *J Bacteriol* 85:541–548. <https://doi.org/10.1128/JB.85.3.541-548.1963>.
 61. Whang H, Neter E. 1963. Study of heterogenetic (Kunin) antibodies in serum of healthy subjects and children with enteric and urinary tract infections. *J Pediatr* 63:412–419. [https://doi.org/10.1016/S0022-3476\(63\)80429-1](https://doi.org/10.1016/S0022-3476(63)80429-1).
 62. Moncevičiūtė-Eringienė E, Kazbarienė B, Milašienė V, Characiejus D, Kemeklienė R. 2006. Natural antibodies to endotoxin in experimental and clinical oncology. *Exp Oncol* 28:89–91.
 63. Gorzynski EA, Ambrus JL, Neter E. 1971. Effect of common enterobacterial antiserum on experimental *Salmonella* Typhimurium infection of mice. *Proc Soc Exp Biol Med* 137:1209–1212. <https://doi.org/10.3181/00379727-137-35757>.
 64. Gorzynski EA, Krasny SA. 1975. Immunologic mimicry between mouse tissue and enterobacterial common antigen. *Immunol Comm* 4:39–49. <https://doi.org/10.3109/08820137509055760>.
 65. Gorzynski EA, Neter E, Ambrus JL. 1970. Differences in antibody responses of mouse strains to enterobacterial common antigen. *Proc Soc*

- Exp Biol Med 134:776–779. <https://doi.org/10.3181/00379727-134-34881>.
66. Dlaz F, Neter E. 1968. Antibody response to the common enterobacterial antigen of children with shigellosis, salmonellosis or urinary tract infection. *Am J Med Sci* 256:18–24. <https://doi.org/10.1097/00000441-196807000-00003>.
 67. Neter E, Whang H, Nowotny A. 1972. The common antigen of Gram-negative bacteria. Cellular antigens. Springer-Verlag, New York, NY.
 68. Andersen HJ. 1966. Studies of urinary tract infections in infancy and childhood: VII. The relation of *E. coli* antibodies in pyelonephritis as measured by homologous and common (Kunin) antigens. *J Pediatr* 68:542–550. [https://doi.org/10.1016/S0022-3476\(66\)80391-8](https://doi.org/10.1016/S0022-3476(66)80391-8).
 69. Saito I. 1967. Serological study of chronic pyelonephritis, especially on the diagnostic value of the estimation of enterobacterial common antibody response. *Fukushima J Med Sci* 14:45–53.
 70. Domingue G, Salhi A, Rountree C, Little W. 1970. Prevention of experimental hematogenous and retrograde pyelonephritis by antibodies against enterobacterial common antigen. *Infect Immun* 2:175–182. <https://doi.org/10.1128/IAI.2.2.175-182.1970>.
 71. Frentz G, Domingue G. 1973. Effects of immunization with ethanol-soluble enterobacterial common antigen on *in vivo* bacterial clearance and hematogenous pyelonephritis. *Proc Soc Exp Biol Med* 142:246–252. <https://doi.org/10.3181/00379727-142-36998>.
 72. McLaughlin JC, Domingue G. 1974. The immunologic role of the ethanol-soluble enterobacterial common antigen versus experimental renal infection. *Immunol Invest* 3:51–75. <https://doi.org/10.3109/08820137409055745>.
 73. Duda KT. 2007. Reactivity of polyclonal antisera against R mutants of *Yersinia enterocolitica* O:3. Dissertation. University of Silesia, Katowice, Poland.
 74. Radziejewska-Lebrecht J, Skurnik M, Shashkov AS, Brade L, Różalski A, Bartodziejska B, Mayer H. 1998. Immunochemical studies on R mutants of *Yersinia enterocolitica* O:3. *Acta Biochim Pol* 45:1011–1019. https://doi.org/10.18388/abp.1998_4358.
 75. Radziejewska-Lebrecht J, Kasperkiewicz K, Skurnik M, Brade L, Steinmetz I, Świerzek AS, Muszyński A. 2004. ECA-antibodies in antisera against R mutants of *Yersinia enterocolitica* O:3, p 215–218. The genus *Yersinia*. Springer, New York, NY.
 76. Duda KA, Duda KT, Beczała A, Kasperkiewicz K, Radziejewska-Lebrecht J, Skurnik M. 2009. ECA-immunogenicity of *Proteus mirabilis* strains. *Arch Immunol Ther Exp (Warsz)* 57:147–151. <https://doi.org/10.1007/s00005-009-0018-9>.
 77. Rastawicki W. 2007. Humoral response to selected antigens of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in the course of yersiniosis in humans. I. Occurrence of antibodies to enterobacterial common antigen (ECA). *Med Dosw Mikrobiol* 59:93–102.
 78. McCabe WR, Greely A. 1973. Common enterobacterial antigen II. Effect of immunization on challenge with heterologous *Bacilli*. *Infect Immun* 7:386–392. <https://doi.org/10.1128/IAI.7.3.386-392.1973>.
 79. Albertson TE, Panacek EA, MacArthur RD, Johnson SB, Benjamin E, Matuschak GM, Zaloga G, Maki D, Silverstein J, Tobias JK. 2003. Multi-center evaluation of a human monoclonal antibody to *Enterobacteriaceae* common antigen in patients with Gram-negative sepsis. *Crit Care Med* 31:419–427. <https://doi.org/10.1097/01.CCM.0000045564.51812.3F>.
 80. Bridge DR, Whitmire JM, Gilbreath JJ, Metcalf ES, Merrell DS. 2015. An enterobacterial common antigen mutant of *Salmonella enterica* serovar Typhimurium as a vaccine candidate. *Int J Med Microbiol* 305:511–522. <https://doi.org/10.1016/j.ijmm.2015.05.004>.
 81. Huang C, Liu Q, Luo Y, Li P, Liu Q, Kong Q. 2016. Regulated delayed synthesis of lipopolysaccharide and enterobacterial common antigen of *Salmonella* Typhimurium enhances immunogenicity and cross-protective efficacy against heterologous *Salmonella* challenge. *Vaccine* 34:4285–4292. <https://doi.org/10.1016/j.vaccine.2016.07.010>.
 82. Aoki S, Yoshikawa K, Yokoyama T, Nonogaki T, Iwasaki S, Mitsui T, Niwa S. 1996. Role of enteric bacteria in the pathogenesis of rheumatoid arthritis: evidence for antibodies to enterobacterial common antigens in rheumatoid sera and synovial fluids. *Ann Rheum Dis* 55:363–369. <https://doi.org/10.1136/ard.55.6.363>.
 83. Aoki S, Mitsui T. 2005. Histological features of rheumatoid arthritis patients having antibodies to enterobacterial common antigens: correlation of antibody levels with semiquantitative histologic scores and laboratory markers. *Clin Exp Rheumatol* 23:8–13.
 84. Rashid T, Jayakumar K, Binder A, Ellis S, Cunningham P, Ebringer A. 2007. Rheumatoid arthritis patients have elevated antibodies to cross-reactive and non-cross-reactive antigens from *Proteus* microbes. *Clin Exp Rheumatol* 25:259–267.
 85. Basu S, Kuhn HM, Neszemelyi A, Himmelspach K, Mayer H. 1987. Chemical characterization of enterobacterial common antigen isolated from *Plesiomonas shigelloides* ATCC 14029. *Eur J Biochem* 162:75–81. <https://doi.org/10.1111/j.1432-1033.1987.tb10544.x>.
 86. Vinogradov EV, Knirel YA, Thomas-Oates JE, Shashkov AS, L'vov VL. 1994. The structure of the cyclic enterobacterial common antigen (ECA) from *Yersinia pestis*. *Carbohydr Res* 258:223–232. [https://doi.org/10.1016/0008-6215\(94\)84088-1](https://doi.org/10.1016/0008-6215(94)84088-1).
 87. Erbel PJ, Barr K, Gao N, Gerwig GJ, Rick PD, Gardner KH. 2003. Identification and biosynthesis of cyclic enterobacterial common antigen in *Escherichia coli*. *J Bacteriol* 185:1995–2004. <https://doi.org/10.1128/JB.185.6.1995-2004.2003>.
 88. Kajimura J, Rahman A, Rick PD. 2005. Assembly of cyclic enterobacterial common antigen in *Escherichia coli* K-12. *J Bacteriol* 187:6917–6927. <https://doi.org/10.1128/JB.187.20.6917-6927.2005>.
 89. Männel D, Mayer H. 1978. Isolation and chemical characterization of the enterobacterial common antigen. *Eur J Biochem* 86:361–370. <https://doi.org/10.1111/j.1432-1033.1978.tb12318.x>.
 90. Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* 277:1453–1462. <https://doi.org/10.1126/science.277.5331.1453>.
 91. Barr K, Klena J, Rick PD. 1999. The modality of enterobacterial common antigen polysaccharide chain lengths is regulated by α_{349} of the *wec* gene cluster of *Escherichia coli* K-12. *J Bacteriol* 181:6564–6568. <https://doi.org/10.1128/JB.181.20.6564-6568.1999>.
 92. Rahman A, Barr K, Rick PD. 2001. Identification of the structural gene for the TDP-Fuc4NAc:lipid II Fuc4NAc transferase involved in synthesis of enterobacterial common antigen in *Escherichia coli* K-12. *J Bacteriol* 183:6509–6516. <https://doi.org/10.1128/JB.183.22.6509-6516.2001>.
 93. Rick P, Silver R. 1996. Enterobacterial common antigen and capsular polysaccharides, p 104–122. *Escherichia coli and Salmonella: cellular and molecular biology*, 2nd ed. ASM Press, Washington, DC.
 94. Daniels DL, Plunkett G, Burland V, Blattner FR. 1992. Analysis of the *Escherichia coli* genome: DNA sequence of the region from 84.5 to 86.5 minutes. *Science* 257:771–778. <https://doi.org/10.1126/science.1379743>.
 95. Dodgson C, Amor P, Whitfield C. 1996. Distribution of the *rol* gene encoding the regulator of lipopolysaccharide O-chain length in *Escherichia coli* and its influence on the expression of group I capsular K antigens. *J Bacteriol* 178:1895–1902. <https://doi.org/10.1128/JB.178.7.1895-1902.1996>.
 96. Franco AV, Liu D, Reeves PR. 1996. A Wzz (Cld) protein determines the chain length of K lipopolysaccharide in *Escherichia coli* O8 and O9 strains. *J Bacteriol* 178:1903–1907. <https://doi.org/10.1128/JB.178.7.1903-1907.1996>.
 97. Whitfield C. 1995. Biosynthesis of lipopolysaccharide O antigens. *Trends Microbiol* 3:178–185. [https://doi.org/10.1016/S0966-842X\(00\)88917-9](https://doi.org/10.1016/S0966-842X(00)88917-9).
 98. Whitfield C, Amor PA, Köplin R. 1997. Modulation of the surface architecture of Gram-negative bacteria by the action of surface polymer: lipid A–core ligase and by determinants of polymer chain length. *Mol Microbiol* 23:629–638. <https://doi.org/10.1046/j.1365-2958.1997.2571614.x>.
 99. Jones MB, Rosenberg JN, Betenbaugh MJ, Krag SS. 2009. Structure and synthesis of polyisoprenoids used in N-glycosylation across the three domains of life. *Biochim Biophys Acta Gen Subj* 1790:485–494. <https://doi.org/10.1016/j.bbagen.2009.03.030>.
 100. Barr K, Nunes-Edwards P, Rick PD. 1989. *In vitro* synthesis of a lipid-linked trisaccharide involved in synthesis of enterobacterial common antigen. *J Bacteriol* 171:1326–1332. <https://doi.org/10.1128/JB.171.3.1326-1332.1989>.
 101. Barr K, Rick P. 1987. Biosynthesis of enterobacterial common antigen in *Escherichia coli*. *In vitro* synthesis of lipid-linked intermediates. *J Biol Chem* 262:7142–7150.
 102. Rick P, Mayer H, Neumeyer B, Wolski S, Bitter-Suermann D. 1985. Biosynthesis of enterobacterial common antigen. *J Bacteriol* 162:494–503. <https://doi.org/10.1128/JB.162.2.494-503.1985>.
 103. Meier-Dieter U, Starman R, Barr K, Mayer H, Rick P. 1990. Biosynthesis of enterobacterial common antigen in *Escherichia coli*. *Biochemical char-*

- acterization of Tn10 insertion mutants defective in enterobacterial common antigen synthesis. *J Biol Chem* 265:13490–13497.
104. Samuel G, Reeves P. 2003. Biosynthesis of O-antigens: genes and pathways involved in nucleotide sugar precursor synthesis and O-antigen assembly. *Carbohydr Res* 338:2503–2519. <https://doi.org/10.1016/j.carres.2003.07.009>.
 105. Whitfield C. 2006. Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. *Annu Rev Biochem* 75:39–68. <https://doi.org/10.1146/annurev.biochem.75.103004.142545>.
 106. Brown ED, Vivas EI, Walsh CT, Kolter R. 1995. MurA (MurZ), the enzyme that catalyzes the first committed step in peptidoglycan biosynthesis, is essential in *Escherichia coli*. *J Bacteriol* 177:4194–4197. <https://doi.org/10.1128/JB.177.14.4194-4197.1995>.
 107. Typas A, Banzhaf M, Gross CA, Vollmer W. 2012. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat Rev Microbiol* 10:123–136. <https://doi.org/10.1038/nrmicro2677>.
 108. Rick P, Fung L, Ho C, Osborn M. 1977. Lipid A mutants of *Salmonella Typhimurium*. Purification and characterization of a lipid A precursor produced by a mutant in 3-deoxy-D-manno-octulosonate-8-phosphate synthetase. *J Biol Chem* 252:4904–4912.
 109. Amer AO, Valvano MA. 2000. The N-terminal region of the *Escherichia coli* WecA (Rfe) protein, containing three predicted transmembrane helices, is required for function but not for membrane insertion. *J Bacteriol* 182:498–503. <https://doi.org/10.1128/JB.182.2.498-503.2000>.
 110. Lehrer J, Vigeant KA, Tatar LD, Valvano MA. 2007. Functional characterization and membrane topology of *Escherichia coli* WecA, a sugar-phosphate transferase initiating the biosynthesis of enterobacterial common antigen and O-antigen lipopolysaccharide. *J Bacteriol* 189:2618–2628. <https://doi.org/10.1128/JB.01905-06>.
 111. Kajimura J, Rahman A, Hsu J, Evans MR, Gardner KH, Rick PD. 2006. O acetylation of the enterobacterial common antigen polysaccharide is catalyzed by the product of the *yiaH* gene of *Escherichia coli* K-12. *J Bacteriol* 188:7542–7550. <https://doi.org/10.1128/JB.00783-06>.
 112. Sala RF, Morgan PM, Tanner ME. 1996. Enzymatic formation and release of a stable glycol intermediate: the mechanism of the reaction catalyzed by UDP-N-acetylglucosamine 2-epimerase. *J Am Chem Soc* 118:3033–3034. <https://doi.org/10.1021/ja960266z>.
 113. Morgan PM, Sala RF, Tanner ME. 1997. Eliminations in the reactions catalyzed by UDP-N-acetylglucosamine 2-epimerase. *J Am Chem Soc* 119:10269–10277. <https://doi.org/10.1021/ja971718q>.
 114. Campbell RE, Mosimann SC, Tanner ME, Strynadka NC. 2000. The structure of UDP-N-acetylglucosamine 2-epimerase reveals homology to phosphoglycosyl transferases. *Biochemistry* 39:14993–15001. <https://doi.org/10.1021/bi001627x>.
 115. Samuel J, Tanner ME. 2004. Active site mutants of the “non-hydrolyzing” UDP-N-acetylglucosamine 2-epimerase from *Escherichia coli*. *Biochim Biophys Acta Proteom* 1700:85–91. <https://doi.org/10.1016/j.bbapap.2004.03.017>.
 116. Kawamura T, Ishimoto N, Ito E. 1979. Enzymatic synthesis of uridine diphosphate N-acetyl-D-mannosaminuronic acid. *J Biol Chem* 254:8457–8465. [https://doi.org/10.1016/0014-5793\(74\)80013-X](https://doi.org/10.1016/0014-5793(74)80013-X).
 117. Barr K, Ward S, Meier-Dieter U, Mayer H, Rick P. 1988. Characterization of an *Escherichia coli* *rff* mutant defective in transfer of N-acetylmannosaminuronic acid (ManNAcA) from UDP-ManNAcA to a lipid-linked intermediate involved in enterobacterial common antigen synthesis. *J Bacteriol* 170:228–233. <https://doi.org/10.1128/JB.170.1.228-233.1988>.
 118. Marolda CL, Valvano MA. 1995. Genetic analysis of the dTDP-rhamnose biosynthesis region of the *Escherichia coli* VW187 (O7: K1) *rff* gene cluster: identification of functional homologs of *rffB* and *rffA* in the *rff* cluster and correct location of the *rffE* gene. *J Bacteriol* 177:5539–5546. <https://doi.org/10.1128/JB.177.19.5539-5546.1995>.
 119. Meier-Dieter U, Barr K, Starman R, Hatch L, Rick P. 1992. Nucleotide sequence of the *Escherichia coli* *rfe* gene involved in the synthesis of enterobacterial common antigen. Molecular cloning of the *rfe-rff* gene cluster. *J Biol Chem* 267:746–753.
 120. Sivaraman J, Sauv e V, Matte A, Cygler M. 2002. Crystal structure of *Escherichia coli* glucose-1-phosphate thymidyltransferase (RffH) complexed with dTTP and Mg²⁺. *J Biol Chem* 277:44214–44219. <https://doi.org/10.1074/jbc.M206932200>.
 121. Gross JW, Hegeman AD, Gerrata B, Frey PA. 2001. Dehydration is catalyzed by glutamate-136 and aspartic acid-135 active site residues in *Escherichia coli* dTDP-glucose 4, 6-dehydratase. *Biochemistry* 40:12497–12504. <https://doi.org/10.1021/bi011133c>.
 122. Gerrata B, Cleland WW, Frey PA. 2001. Mechanistic roles of Thr134, Tyr160, and Lys 164 in the reaction catalyzed by dTDP-glucose 4, 6-dehydratase. *Biochemistry* 40:9187–9195. <https://doi.org/10.1021/bi0108249>.
 123. Hegeman AD, Gross JW, Frey PA. 2001. Probing catalysis by *Escherichia coli* dTDP-glucose-4, 6-dehydratase: identification and preliminary characterization of functional amino acid residues at the active site. *Biochemistry* 40:6598–6610. <https://doi.org/10.1021/bi010441a>.
 124. Hwang BY, Lee HJ, Yang YH, Joo HS, Kim BG. 2004. Characterization and investigation of substrate specificity of the sugar aminotransferase WecE from *E. coli* K12. *Chem Biol* 11:915–925. <https://doi.org/10.1016/j.chembiol.2004.04.015>.
 125. Wang F, Singh S, Xu W, Helmich KE, Miller MD, Cao H, Craig AB, Jon ST, Phillips GN, Jr. 2015. Structural basis for the stereochemical control of amine installation in nucleotide sugar aminotransferases. *ACS Chem Biol* 10:2048–2056. <https://doi.org/10.1021/acscmbio.5b00244>.
 126. Hung MN, Rangarajan E, Munger C, Nadeau G, Sulea T, Matte A. 2006. Crystal structure of TDP-fucosamine acetyltransferase (WecD) from *Escherichia coli*, an enzyme required for enterobacterial common antigen synthesis. *J Bacteriol* 188:5606–5617. <https://doi.org/10.1128/JB.00306-06>.
 127. Macpherson DF, Manning PA, Morona R. 1995. Genetic analysis of the *rffB* gene of *Shigella flexneri*. *Gene* 155:9–17. [https://doi.org/10.1016/0378-1119\(94\)00918-1](https://doi.org/10.1016/0378-1119(94)00918-1).
 128. Rick PD, Barr K, Sankaran K, Kajimura J, Rush JS, Waechter CJ. 2003. Evidence that the *wzxE* gene of *Escherichia coli* K-12 encodes a protein involved in the transbilayer movement of a trisaccharide-lipid intermediate in the assembly of enterobacterial common antigen. *J Biol Chem* 278:16534–16542. <https://doi.org/10.1074/jbc.M301750200>.
 129. Paulsen IT, Beness AM, Saier MH. 1997. Computer-based analyses of the protein constituents of transport systems catalysing export of complex carbohydrates in bacteria. *Microbiology* 143:2685–2699. <https://doi.org/10.1099/00221287-143-8-2685>.
 130. Marolda CL, Tatar LD, Alaimo C, Aebi M, Valvano MA. 2006. Interplay of the Wzx translocase and the corresponding polymerase and chain length regulator proteins in the translocation and periplasmic assembly of lipopolysaccharide O antigen. *J Bacteriol* 188:5124–5135. <https://doi.org/10.1128/JB.00461-06>.
 131. Nath P, Morona R. 2015. Detection of Wzy/Wzx interaction in *Shigella flexneri*. *Microbiology* 161:1797–1805. <https://doi.org/10.1099/mic.0.000132>.
 132. Morona R, Van Den Bosch L, Daniels C. 2000. Evaluation of Wzz/MPA1/MPA2 proteins based on the presence of coiled-coil regions. *Microbiology* 146:1–4. <https://doi.org/10.1099/00221287-146-1-1>.
 133. Tocilj A, Munger C, Proteau A, Morona R, Purins L, Ajamian E, Wagner J, Papadopoulos M, Van Den Bosch L, Rubinstein JL, F ethi ere J, Matte A, Cygler M. 2008. Bacterial polysaccharide co-polymerases share a common framework for control of polymer length. *Nat Struct Mol Biol* 15:130–138. <https://doi.org/10.1038/nsmb.1374>.
 134. Larue K, Kimber MS, Ford R, Whitfield C. 2009. Biochemical and structural analysis of bacterial O-antigen chain length regulator proteins reveals a conserved quaternary structure. *J Biol Chem* 284:7395–7403. <https://doi.org/10.1074/jbc.M809068200>.
 135. Kalynych S, Yao D, Magee J, Cygler M. 2012. Structural characterization of closely related O-antigen lipopolysaccharide (LPS) chain length regulators. *J Biol Chem* 287:15696–15705. <https://doi.org/10.1074/jbc.M112.354837>.
 136. Kalynych S, Cherney M, Bostina M, Rouiller I, Cygler M. 2015. Quaternary structure of WzzB and WzzE polysaccharide copolymerases. *Protein Sci* 24:58–69. <https://doi.org/10.1002/pro.2586>.
 137. Collins RF, Kargas V, Clarke BR, Siebert CA, Clare DK, Bond PJ, Whitfield C, Ford RC. 2017. Full-length, oligomeric structure of Wzz determined by cryo-electron microscopy reveals insights into membrane-bound states. *Structure* 25:806–815. <https://doi.org/10.1016/j.str.2017.03.017>.
 138. Bastin DA, Stevenson G, Brown PK, Haase A, Reeves PR. 1993. Repeat unit polysaccharides of bacteria: a model for polymerization resembling that of ribosomes and fatty acid synthetase, with a novel mechanism for determining chain length. *Mol Microbiol* 7:725–734. <https://doi.org/10.1111/j.1365-2958.1993.tb01163.x>.
 139. Morona R, van den Bosch L, Manning PA. 1995. Molecular, genetic, and topological characterization of O-antigen chain length regulation in *Shigella flexneri*. *J Bacteriol* 177:1059–1068. <https://doi.org/10.1128/JB.177.4.1059-1068.1995>.
 140. Lugowski C, Romanowska E. 1978. Enterobacterial common antigen: isolation from *Shigella sonnei*, purification and immunochemical char-

- acterization. *Eur J Biochem* 91:89–97. <https://doi.org/10.1111/j.1432-1033.1978.tb20941.x>.
141. Muszyński A, Rabsztyń K, Knapska K, Duda KA, Duda-Grychtoł K, Kasperkiewicz K, Radziejewska-Lebrecht J, Holst O, Skurnik M. 2013. Enterobacterial common antigen and O-specific polysaccharide coexist in the lipopolysaccharide of *Yersinia enterocolitica* serotype O:3. *Microbiology* 159:1782–1793. <https://doi.org/10.1099/mic.0.066662-0>.
 142. Staaf M, Höög C, Stevansson B, Maliniak A, Widmalm G. 2001. Conformational investigation of a cyclic enterobacterial common antigen employing NMR spectroscopy and molecular dynamics simulations. *Biochemistry* 40:3623–3628. <https://doi.org/10.1021/bi002282l>.
 143. Färnäck M, Eriksson L, Senchenkova S, Zych K, Knirel YA, Sidorczyk Z, Widmalm G. 2003. Crystal structure of a cyclic enterobacterial common antigen. *Angew Chem Int Ed Engl* 42:2543–2546. <https://doi.org/10.1002/anie.200351250>.
 144. Andersson A, Ahl Å, Eklund R, Widmalm G, Mäler L. 2005. Dynamics in the cyclic enterobacterial common antigen as studied by ¹³C NMR relaxation. *J Biomol NMR* 31:311–320. <https://doi.org/10.1007/s10858-005-1605-7>.
 145. Mitchell AM, Srikumar T, Silhavy TJ. 2018. Cyclic enterobacterial common antigen maintains the outer membrane permeability barrier of *Escherichia coli* in a manner controlled by YhdP. *mBio* 9:e01321-18. <https://doi.org/10.1128/mBio.01321-18>.
 146. Pazos M, Peters K. 2019. Peptidoglycan, p 127–168. Bacterial cell walls and membranes. Springer, New York, NY.
 147. Kalynych S, Morona R, Cygler M. 2014. Progress in understanding the assembly process of bacterial O-antigen. *FEMS Microbiol Rev* 38:1048–1065. <https://doi.org/10.1111/1574-6976.12070>.
 148. Wright A, Dankert M, Fennessey P, Robbins P. 1967. Characterization of a polyisoprenoid compound functional in O-antigen biosynthesis. *Proc Natl Acad Sci U S A* 57:1798–1803. <https://doi.org/10.1073/pnas.57.6.1798>.
 149. Rick PD, Hubbard GL, Kitaoka M, Nagaki H, Kinoshita T, Dowd S, Simplaceanu V, Ho C. 1998. Characterization of the lipid-carrier involved in the synthesis of enterobacterial common antigen (ECA) and identification of a novel phosphoglyceride in a mutant of *Salmonella* Typhimurium defective in ECA synthesis. *Glycobiology* 8:557–567. <https://doi.org/10.1093/glycob/8.6.557>.
 150. Johnson JG, Wilson DB. 1977. Role of a sugar-lipid intermediate in colanic acid synthesis by *Escherichia coli*. *J Bacteriol* 129:225–236. <https://doi.org/10.1128/JB.129.1.225-236.1977>.
 151. Ikeda M, Wachi M, Jung H, Ishino F, Matsuhashi M. 1991. The *Escherichia coli* *mraY* gene encoding UDP-N-acetylmuramoyl-pentapeptide: undecaprenyl-phosphate phospho-N-acetylmuramoyl-pentapeptide transferase. *J Bacteriol* 173:1021–1026. <https://doi.org/10.1128/JB.173.3.1021-1026.1991>.
 152. Tytgat HL, Lebeer S. 2014. The sweet tooth of bacteria: common themes in bacterial glycoconjugates. *Microbiol Mol Biol Rev* 78:372–417. <https://doi.org/10.1128/MMBR.00007-14>.
 153. Jorgenson MA, Young KD. 2016. Interrupting biosynthesis of O antigen or the lipopolysaccharide core produces morphological defects in *Escherichia coli* by sequestering undecaprenyl phosphate. *J Bacteriol* 198:3070–3079. <https://doi.org/10.1128/JB.00550-16>.
 154. Rick PD, Wolski S, Barr K, Ward S, Ramsay-Sharer L. 1988. Accumulation of a lipid-linked intermediate involved in enterobacterial common antigen synthesis in *Salmonella* Typhimurium mutants lacking dTDP-glucose pyrophosphorylase. *J Bacteriol* 170:4008–4014. <https://doi.org/10.1128/JB.170.9.4008-4014.1988>.
 155. Ramos-Morales F, Prieto AI, Beuzón CR, Holden DW, Casadesús J. 2003. Role for *Salmonella enterica* enterobacterial common antigen in bile resistance and virulence. *J Bacteriol* 185:5328–5332. <https://doi.org/10.1128/JB.185.17.5328-5332.2003>.
 156. Danese PN, Oliver GR, Barr K, Bowman GD, Rick PD, Silhavy TJ. 1998. Accumulation of the enterobacterial common antigen lipid II biosynthetic intermediate stimulates *degP* transcription in *Escherichia coli*. *J Bacteriol* 180:5875–5884. <https://doi.org/10.1128/JB.180.22.5875-5884.1998>.
 157. Castelli ME, Fedrigo GV, Clementín AL, Ielmini MV, Feldman MF, Vécovi EG. 2008. Enterobacterial common antigen integrity is a checkpoint for flagellar biogenesis in *Serratia marcescens*. *J Bacteriol* 190:213–220. <https://doi.org/10.1128/JB.01348-07>.
 158. Castelli ME, Vécovi EG. 2011. The Rcs signal transduction pathway is triggered by enterobacterial common antigen structure alterations in *Serratia marcescens*. *J Bacteriol* 193:63–74. <https://doi.org/10.1128/JB.00839-10>.
 159. Jorgenson MA, Kannan S, Laubacher ME, Young KD. 2016. Dead-end intermediates in the enterobacterial common antigen pathway induce morphological defects in *Escherichia coli* by competing for undecaprenyl phosphate. *Mol Microbiol* 100:1–14. <https://doi.org/10.1111/mmi.13284>.
 160. Jann K, Kanegasaki S, Goldemann G, Mäkelä PH. 1979. On the effect of *rfe* mutation on the biosynthesis of the O8 and O9 antigens of *E. coli*. *Biochem Biophys Res Commun* 86:1185–1191. [https://doi.org/10.1016/0006-291X\(79\)90242-0](https://doi.org/10.1016/0006-291X(79)90242-0).
 161. Mäkelä PH, Mayer H, Whang H, Neter E. 1974. Participation of lipopolysaccharide genes in the determination of the enterobacterial common antigen: analysis of R mutants of *Salmonella minnesota*. *J Bacteriol* 119:760–764. <https://doi.org/10.1128/JB.119.3.760-764.1974>.
 162. Mäkelä PH, Jahkola M, Lüderitz O. 1970. A new gene cluster *rfe* concerned with the biosynthesis of *Salmonella* lipopolysaccharide. *J Gen Microbiol* 60:91–106. <https://doi.org/10.1099/00221287-60-1-91>.
 163. Stocker BAD, Mäkelä P. 1978. Genetics of the (Gram-negative) bacterial surface. *Proc R Soc Lond B Biol Sci* 202:5–30. <https://doi.org/10.1098/rspb.1978.0055>.
 164. Rick PD, Hubbard GL, Barr K. 1994. Role of the *rfe* gene in the synthesis of the O8 antigen in *Escherichia coli* K-12. *J Bacteriol* 176:2877–2884. <https://doi.org/10.1128/JB.176.10.2877-2884.1994>.
 165. Hong Y, Liu MA, Reeves PR. 2017. Progress in our understanding of Wzx flippase for translocation of bacterial membrane lipid-linked oligosaccharide. *J Bacteriol* 200:e00154-17. <https://doi.org/10.1128/JB.00154-17>.
 166. Liu MA, Morris P, Reeves PR. 2019. Wzx flippases exhibiting complex O-unit preferences require a new model for Wzx-substrate interactions. *Microbiology Open* 8:e00655. <https://doi.org/10.1002/mbo3.655>.
 167. Li GW, Burkhardt D, Gross C, Weissman JS. 2014. Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. *Cell* 157:624–635. <https://doi.org/10.1016/j.cell.2014.02.033>.
 168. Feldman MF, Marolda CL, Monteiro MA, Perry MB, Parodi AJ, Valvano MA. 1999. The activity of a putative polyisoprenol-linked sugar translocase (Wzx) involved in *Escherichia coli* O antigen assembly is independent of the chemical structure of the O repeat. *J Biol Chem* 274:35129–35138. <https://doi.org/10.1074/jbc.274.49.35129>.
 169. Marolda CL, Vicarioli J, Valvano MA. 2004. Wzx proteins involved in biosynthesis of O antigen function in association with the first sugar of the O-specific lipopolysaccharide subunit. *Microbiology* 150:4095–4105. <https://doi.org/10.1099/mic.0.27456-0>.
 170. Hong Y, Reeves PR. 2014. Diversity of O-antigen repeat unit structures can account for the substantial sequence variation of Wzx translocases. *J Bacteriol* 196:1713–1722. <https://doi.org/10.1128/JB.01323-13>.
 171. Liu MA, Stent TL, Hong Y, Reeves PR. 2015. Inefficient translocation of a truncated O unit by a *Salmonella* Wzx affects both O-antigen production and cell growth. *FEMS Microbiol Lett* 362:fnv053. <https://doi.org/10.1093/femsle/fnv053>.
 172. Hong Y, Cunneen MM, Reeves PR. 2012. The Wzx translocases for *Salmonella enterica* O-antigen processing have unexpected serotype specificity. *Mol Microbiol* 84:620–630. <https://doi.org/10.1111/j.1365-2958.2012.08048.x>.
 173. Klein KA, Fukuto HS, Pelletier M, Romanov G, Grabenstein JP, Palmer LE, Ernst R, Bliska JB. 2012. A transposon site hybridization screen identifies *galU* and *wecBC* as important for survival of *Yersinia pestis* in murine macrophages. *J Bacteriol* 194:653–662. <https://doi.org/10.1128/JB.06237-11>.
 174. Phan MD, Peters KM, Sarkar S, Lukowski SW, Allsopp LP, Moriel DG, Achard ME, Totsika M, Marshall VM, Upton M, Beatson SA, Schembri MA. 2013. The serum resistome of a globally disseminated multidrug resistant uropathogenic *Escherichia coli* clone. *PLoS Genet* 9:e1003834. <https://doi.org/10.1371/journal.pgen.1003834>.
 175. Suezawa C, Yasuda M, Negayama K, Kameyama T, Hirauchi M, Nakai T, Okuda J. 2016. Identification of genes associated with the penetration activity of the human type of *Edwardsiella tarda* EdwGII through human colon epithelial cell monolayers. *Microb Pathog* 95:148–156. <https://doi.org/10.1016/j.micpath.2016.04.007>.
 176. Bohm K, Porwollik S, Chu W, Dover JA, Gilcrease EB, Casjens SR, McClelland M, Parent KN. 2018. Genes affecting progression of bacteriophage P22 infection in *Salmonella* identified by transposon and

- single gene deletion screens. *Mol Microbiol* 108:288–305. <https://doi.org/10.1111/mmi.13936>.
177. Kuhn H, Meier U, Mayer H. 1984. ECA, das gemeinsame antigender enterobacteriaceae stiefkind der mikrobiologie. *Forum Mikrobiol* 7: 274–285.
 178. Barua S, Yamashino T, Hasegawa T, Yokoyama K, Torii K, Ohta M. 2002. Involvement of surface polysaccharides in the organic acid resistance of Shiga Toxin-producing *Escherichia coli* O157: H7. *Mol Microbiol* 43:629–640. <https://doi.org/10.1046/j.1365-2958.2002.02768.x>.
 179. Tamae C, Liu A, Kim K, Sitz D, Hong J, Becket E, Bui A, Solaimani P, Tran KP, Yang H, Miller JH. 2008. Determination of antibiotic hypersensitivity among 4,000 single-gene-knockout mutants of *Escherichia coli*. *J Bacteriol* 190:5981–5988. <https://doi.org/10.1128/JB.01982-07>.
 180. Girgis HS, Hottes AK, Tavazoei S. 2009. Genetic architecture of intrinsic antibiotic susceptibility. *PLoS One* 4:e5629. <https://doi.org/10.1371/journal.pone.0005629>.
 181. Nichols RJ, Sen S, Choo YJ, Beltrao P, Zietek M, Chaba R, Lee S, Kazmierczak KM, Lee KJ, Wong A, Shales M, Lovett S, Winkler ME, Krogan NJ, Typas A, Gross CA. 2011. Phenotypic landscape of a bacterial cell. *Cell* 144:143–156. <https://doi.org/10.1016/j.cell.2010.11.052>.
 182. Gilbreath JJ, Dodds JC, Rick PD, Soloski MJ, Merrell DS, Metcalf ES. 2012. Enterobacterial common antigen mutants of *Salmonella enterica* serovar Typhimurium establish a persistent infection and provide protection against subsequent lethal challenge. *Infect Immun* 80:441–450. <https://doi.org/10.1128/IAI.05559-11>.
 183. McMahon KJ, Castelli ME, Vescovi EG, Feldman MF. 2012. Biogenesis of outer membrane vesicles in *Serratia marcescens* is thermoregulated and can be induced by activation of the Rcs phosphorelay system. *J Bacteriol* 194:3241–3249. <https://doi.org/10.1128/JB.00016-12>.
 184. Kulp AJ, Sun B, Ai T, Manning AJ, Orench-Rivera N, Schmid AK, Kuehn MJ. 2015. Genome-wide assessment of outer membrane vesicle production in *Escherichia coli*. *PLoS One* 10:e0139200. <https://doi.org/10.1371/journal.pone.0139200>.
 185. Saxén H, Hovi M. 1985. The effect of antibodies to the enterobacterial common antigen (ECA) on experimental mouse salmonellosis. *FEMS Microbiol Lett* 27:307–312. <https://doi.org/10.1111/j.1574-6968.1985.tb00687.x>.
 186. Fattom AI, Sarwar J, Basham L, Ennifar S, Naso R. 1998. Antigenic determinants of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharide vaccines. *Infect Immun* 66:4588–4592. <https://doi.org/10.1128/IAI.66.10.4588-4592.1998>.
 187. Jennings HJ, Bhaucharjee AK, Bundle DR, Paul Kenny C, Martin A, Smith IC. 1977. Structures of the capsular polysaccharides of *Neisseria meningitidis* as determined by ¹³C-nuclear magnetic resonance spectroscopy. *J Infect Dis* 136:S78–S83. <https://doi.org/10.1093/infdis/136.Supplement.S78>.
 188. Kooistra O, Lüneberg E, Lindner B, Knirel YA, Frosch M, Zähringer U. 2001. Complex O-acetylation in *Legionella pneumophila* serogroup 1 lipopolysaccharide. Evidence for two genes involved in 8-O-acetylation of legionaminic acid. *Biochemistry* 40:7630–7640. <https://doi.org/10.1021/bi002946r>.
 189. Lemercinier X, Jones C. 1996. Full ¹H NMR assignment and detailed. *Carbohydr Res* 296:83–96. [https://doi.org/10.1016/S0008-6215\(96\)00253-4](https://doi.org/10.1016/S0008-6215(96)00253-4).
 190. Lewis AL, Nizet V, Varki A. 2004. Discovery and characterization of sialic acid O-acetylation in group B *Streptococcus*. *Proc Natl Acad Sci U S A* 101:11123–11128. <https://doi.org/10.1073/pnas.0403010101>.
 191. McNeely TB, Staub JM, Rusk CM, Blum MJ, Donnelly JJ. 1998. Antibody responses to capsular polysaccharide backbone and O-acetate side groups of *Streptococcus pneumoniae* type 9V in humans and rhesus macaques. *Infect Immun* 66:3705–3710. <https://doi.org/10.1128/IAI.66.8.3705-3710.1998>.
 192. Paavonen J, Valtonen V, Kasper D, Malkamäki M, Mäkelä PH. 1981. Serological evidence for the role of *Bacteroides fragilis* and *Enterobacteriaceae* in the pathogenesis of acute pelvic inflammatory disease. *Lancet* 317:293–295. [https://doi.org/10.1016/S0140-6736\(81\)91909-7](https://doi.org/10.1016/S0140-6736(81)91909-7).
 193. Rabsztyń K, Kasperkiewicz K, Duda K, Li C-M, Łukasik M, Radziejewska-Lebrecht J, Skurnik M. 2011. Characterization of anti-ECA antibodies in rabbit antiserum against rough *Yersinia enterocolitica* O:3. *Biochemistry (Mosc)* 76:832–839. <https://doi.org/10.1134/S0006297911070145>.
 194. Adeolu M, Alnajjar S, Naushad S, Gupta RS. 2016. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for *Enterobacteriales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwinaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. *Int J Syst Evol Microbiol* 66:5575–5599. <https://doi.org/10.1099/ijsem.0.001485>.
 195. Gozdziwicz TK, Lugowski C, Lukaszewicz J. 2014. First evidence for a covalent linkage between enterobacterial common antigen and lipopolysaccharide in *Shigella sonnei* phase II ECALPS. *J Biol Chem* 289: 2745–2754. <https://doi.org/10.1074/jbc.M113.512749>.
 196. Johns MA, Whiteside RE, Baker EE, McCabe WR. 1973. Common enterobacterial antigen: I. Isolation and purification from *Salmonella typhosa* 0:901. *J Immunol* 110:781–790.
 197. Kuhn HM, Basu S, Mayer H. 1987. Comparison of enterobacterial common antigen from different species by serological techniques. *Eur J Biochem* 162:69–74. <https://doi.org/10.1111/j.1432-1033.1987.tb10543.x>.
 198. Fregolino E, Ivanova R, Lanzetta R, Molinaro A, Parrilli M, Paunova-Krasteva T, Stoitsova SR, De Castro C. 2012. Occurrence and structure of cyclic enterobacterial common antigen in *Escherichia coli* O157:H⁻. *Carbohydr Res* 363:29–32. <https://doi.org/10.1016/j.carres.2012.09.017>.
 199. Marx A, Petcovič M. 1975. Immunochemical studies on purified common enterobacterial antigen (KUNIN). *Zentralbl Bakteriol Orig A* 233: 486–494.
 200. Hammarström S, Carlsson HE, Perlmann P, Svensson S. 1971. Immunochemistry of the common antigen of *Enterobacteriaceae* (Kunin) relation to lipopolysaccharide core structure. *J Exp Med* 134:565–576. <https://doi.org/10.1084/jem.134.3.565>.
 201. Peters H, Jürs M, Jann B, Jann K, Timmis KN, Bitter-Suermann D. 1985. Monoclonal antibodies to enterobacterial common antigen and to *Escherichia coli* lipopolysaccharide outer core: demonstration of an antigenic determinant shared by enterobacterial common antigen and *E. coli* K5 capsular polysaccharide. *Infect Immun* 50:459–466. <https://doi.org/10.1128/IAI.50.2.459-466.1985>.