The presence of these pre-atherosclerotic lesions in the proximal aorta demonstrates that a simple lack of apoE is sufficient to initiate atherogenesis. Although different strains of mice exhibit heritable differences in the levels of their circulating lipids (*15*), no inbred strains are known to develop lesions spontaneously on a normal diet. However, they do show significant differences in their tendencies to form atherosclerotic plaques when placed on a high fat diet (*16*). Strain C57BL/6 is more susceptible to atherogenic diets than others that have been tested and strain 129/J is moderately susceptible (*16*). The apoE-deficient mice we studied here are mainly F2 animals that have a combination of the genetic backgrounds of both C57BL/6 and 129/J; the precise composition is unique for each individual animal (*17*). This genetic heterogeneity may cause some variability among different individuals in their plasma lipoprotein phenotypes and in the likelihood of their developing arterial lesions. However, all 40 mice lacking apoE had elevated total plasma cholesterol levels, and all six that we have studied histologically had lesions in their proximal aorta. None of these changes were observed in normal and heterozygous litter mates.

The early development of lesions in mice lacking apoE makes them of great practical value. The combined phenotype of the homozygotes (high cholesterol and early development of non-lethal lesions) provides a baseline against which either detrimental or protective genetic and environmental factors can be investigated. These mice should also be of value for use as an in vivo test system for studies of pharmacological or genetic treatments of hyperlipidemia.

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14. After overnight fast, mice were killed with an overdose of avertin (2,2,2-tribromoethanol) and the vascular tree was perfused via the heart with 4% (w/v) paraformaldehyde in 0.12 M phosphate buffer, pH 7.4. The heart and entire aorta, including the iliacs, were removed intact and immersed in fresh paraformaldehyde. Prior to cryostat sectioning, the heart and an approximately 0.5-cm length of ascending aorta was removed from the remainder of the aorta. The heart was divided into two halves by cutting transversely along a line parallel to the tip of the atria. The half with the attached aortic segment was then placed in OCT compound (Tissue-Tex), gently compressed to remove bubbles, positioned in semi-frozen OCT to allow cross sectioning of the aorta, and then frozen in OCT compound on a metal pedestal prior to sectioning. Eight micrometer-thick frozen sections at 24-μm intervals were made beginning with the ascending aorta and proceeding through the entire aortic sinus until the ventricular chamber was reached. Adequacy of the frozen sectioning process was monitored by light microscopic evaluation of unstained sections. The frozen sections were allowed to dry overnight at room temperature. Sections were stained to detect lipid with Sudan IVB followed by counter-staining with Harris hematoxylin.
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17. The embryonic stem (ES) cells we used for introducing a modification into *Apoe* were derived from strain 129/Ola. Chimeras made from these ES cells were crossed with strain C57BL/6J mice, and F1 offspring that were heterozygous for the modified gene were obtained. F2 mice homozygous for the mutation were then generated by crossing two F1 heterozygotes. The genetic background of an F2 animal is therefore a discrete blending of the two strains that is unique to each particular animal.
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Formation of a Gated Channel by a Ligand-Specific Transport Protein in the Bacterial Outer Membrane

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The ferric enterobactin receptor (FepA) is a high-affinity ligand-specific transport protein in the outer membrane of Gram-negative bacteria. Deletion of the cell-surface ligand-binding peptides of FepA generated mutant proteins that were incapable of high-affinity uptake but that instead formed nonspecific, passive channels in the outer membrane. Unlike native FepA, these pores acted independently of the accessory protein TonB, which suggests that FepA is a gated porin and that TonB acts as its gatekeeper by facilitating the entry of ligands into the FepA channel. The sequence homology among TonB-dependent proteins suggests that all ligand-specific outer membrane receptors may function by this gated-porin mechanism.

The cell envelope of Gram-negative bacteria contains high-affinity, ligand-specific outer membrane proteins that translocate substrates into the periplasm. Such transport systems are multifunctional—a single outer membrane protein usually serves as the surface receptor for several dissimilar ligands (*1*)—and multicomponent, requiring the participation of periplasmic and cytoplasmic membrane proteins (*2*). TonB is the most notable of these accessory proteins; it resides in the cytoplasmic membrane but is thought to project across the periplasmic space and facilitate the transport function of outer membrane receptors by direct, protein-to-protein interactions (*3*).

Ligand-specific receptor proteins have been considered to be distinct from porins, which form nonspecific, hydrophilic channels in the outer membrane (*4*). Porins contain amphiphilic β strands assembled in the form of a β barrel (*5*), which acts as a molecular sieve that equilibrates small molecules (<600 daltons) across the bilayer

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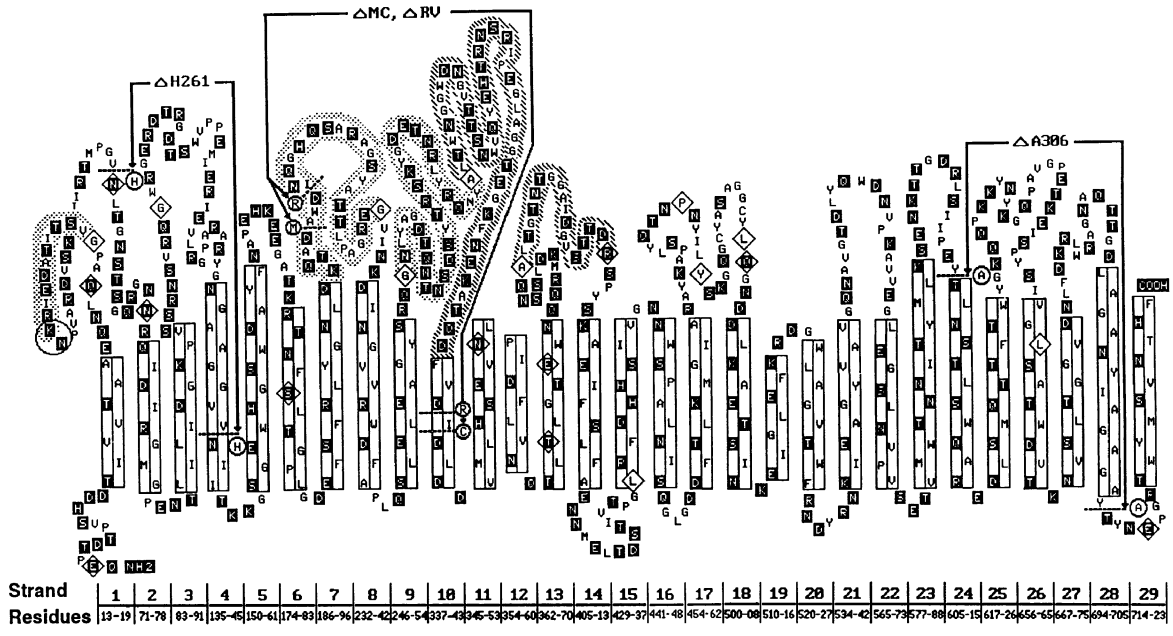

Fig. 1. Location of deletion mutations in the proposed structure of FepA. The predicted structural and topological features of FepA (*7*) are shown. Monoclonal antibody binding to surface epitopes in some regions (striped shading) but not others (stippled shading) blocks ligand recognition. Hydrophilic amino acids are enclosed in black boxes. Vertical boxes designate putative transmembrane β strands, and diamonds indicate sites of FepA::PhoA fusion (*22*). Residues deleted in ΔH261 (*10*), ΔMC (*9*), ΔRV (*7, 9, 10*), and ΔA306 (*10*) are also shown; H, M, C, R, and A shown in circles mark the locations in FepA that correspond to cleavage of *fepA* by Hpa I, Mlu I, Cla I, Eco RV, and Acc I, respectively, which were used for construction of the deletion mutations.

(6). FepA, a ligand-specific, outer membrane protein dependent on TonB, may also contain a β barrel domain because it too has amphiphilic polypeptides that are located in the bilayer (*7*). We have now obtained evidence for the existence of a nonspecific channel within FepA. Our results suggest, however, that unlike known porins, the FepA channel is closed at the cell surface by loops of hydrophilic peptides that selectively bind ligands.

Metal chelates that are too large to diffuse through porins are prototypical substrates for TonB-dependent receptors (*3*). Bacteria secrete catechol or hydroxamate iron chelators, called siderophores, in response to environmental iron deficiency (*8*). The native *Escherichia coli* siderophore, enterobactin, forms a hexacoordinate complex with extracellular Fe^{3+} that enters the cell through FepA (*1*). The 723–amino acid FepA protein contains a surface-exposed region (bounded by residues 258 and 339) that binds ferric enterobactin and colicins B and D (*7*). By deletion mutagenesis, we have removed a segment of approximately 140 amino acids (residues 202 to 340) from this central domain of FepA. The deletions eliminate the ligand-binding domain of the receptor but leave its predicted transmembrane β strands largely intact (Fig. 1); they were created by endonuclease digestion of *fepA* with Mlu I and Cla I (*fepAΔMC*) or Eco RV (*fepAΔRV*) (9). Two additional deletions, *fepAΔH261* and *fepAΔA306* (*9, 10*), that are upstream and downstream, respectively, of the region encoding the central ligand-binding domain (Figs. 1 and 2) were also analyzed.

Siderophore uptake experiments suggest that the *fepAΔMC* and *fepAΔRV* mutations transform the ligand-specific, high-affinity receptor into a nonspecific channel. Neither mutant protein bound ferric enterobactin (Fig. 3A) or colicins, yet both ΔMC and ΔRV (the corresponding mutant receptors) transported ferric enterobactin (Fig. 3B). Unlike wild-type FepA, however, which shows high-affinity saturation kinetics, ΔMC and ΔRV required high concentrations of ferric enterobactin (>5 μM) to function at detectable levels, and their rate of ferric enterobactin uptake was linearly related to its concentration over the range 5 to 100 μM. This linear relation between uptake rate and substrate concentration implies passive diffusion across the outer membrane that is distinct from the high-affinity transport of ferric enterobactin through FepA. The hydrophilic channels of bacterial porins facilitate the passage of small molecules in an identical manner (*4, 6*). ΔH261 and ΔA306, which were expressed from the same plasmid vector at comparable amounts (*11*) and contain deletions that are similar in overall size but remove fewer surface residues than ΔMC and ΔRV (Figs. 1 and 2) (9), did not mediate ferric enterobactin uptake.

Another distinguishing characteristic of porins is their lack of solute specificity (6). ΔMC and ΔRV exhibited similar nonspecificity, as evidenced by their transport of molecules that are structurally unrelated to ferric enterobactin. Although FepA neither binds nor transports hydroxamate sidero-

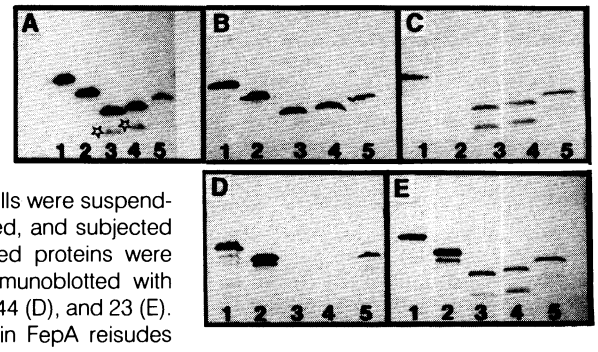

Fig. 2. (A through **E)** Protein immunoblots of FepA deletion mutants. KDF541 (*19*) (Δ*fepA*) harboring *fepA*$^+$ (lanes 1), *fepAΔH261* (lanes 2), *fepAΔMC* (lanes 3), *fepAΔRV* (lanes 4), or *fepAΔA306* (lanes 5) derivatives of pUC18 was grown in MOPSs medium (*23*), and 2 × 10^8 cells were suspended in SDS-PAGE sample buffer, boiled, and subjected to electrophoresis (*7*). The separated proteins were transferred to nitrocellulose and immunoblotted with MAbs to FepA: 41 (A), 29 (B), 11 (C), 44 (D), and 23 (E). MAb 41 recognizes an epitope within FepA reisudes 100 to 178 (*7*); the locations in FepA of the epitopes recognized by the other MAbs are given in Table 2. In (A), bacteria were derived from a typical experiment described in Fig. 3; the immunoblot was developed with ^{125}I-labeled protein A and therefore represents quantitatively the expression of wild-type and mutant FepA proteins. Although some degradation of ΔMC and ΔRV is apparent (starred bands), quantitation by image analysis (Ambis 4000, Ambis Inc., San Diego, California) showed that these products are less than 5% of the total FepA present. The blots shown in (B) through (E) were developed with goat antibodies to mouse immunoglobulin and nitroblue tetrazolium–bromochloroindoyl phosphate (*7*), a more sensitive indicator that identifies the degradation products of FepA present in the outer membrane.

phores, ΔMC and ΔRV mediated the uptake of one such molecule, ferrichrome (8) (Fig. 3C). As with ferric enterobactin, the rate of ferrichrome accumulation was proportional to its concentration over the range 5 to 100 μM. Neither wild-type FepA nor ΔH261 or ΔA306 promoted the uptake of ferrichrome (Fig. 3C). These and other data imply that the *fepA*ΔRV and *fepA*ΔMC mutations eliminate peptides in the ligand-binding domain that normally occlude an underlying channel. The unshielding of this pore ostensibly allows penetration of ferrichrome through the outer membrane. Although *fepAΔH261* and *fepAΔA306* likely also change the architecture of FepA surface peptides, they appear to do so in a manner that does not render the receptor permeable to molecules as large as ferrichrome (740 daltons).

Uptake through the mutant proteins was not limited to siderophores; bacteria carrying *fepA*ΔMC and *fepA*Δ*RV* were susceptible to antibiotics that cannot penetrate the OmpF, OmpC, or PhoE porin channels (Table 1). For example, ΔMC and ΔRV conferred sensitivity to SDS, erythromycin, and bacitracin and increased susceptibility to novobiocin, chloramphenicol, and rifampin. ΔH261 and ΔA306 did not confer susceptibility to the same antibiotics or did so only weakly (*12*).

These data illustrate the characteristic phenotype produced by the deletion of FepA residues 202 to 340 and support the conclusion that the mutant receptors create a pore in the outer membrane. Because it shows no apparent substrate specificity, the FepA channel can be considered a porin (*4–6*). ΔMC and ΔRV create an antibiotic sensitivity profile that is nearly identical to that of *ompF* deletion mutants that increase the effective diameter of the OmpF pore (*13*); the exception is the resistance of bacteria carrying *fepA*ΔMC and *fepA*Δ*RV* to deoxycholate. Sensitivity to SDS but not to deoxycholate is an important contrast, for it shows that bacteria that express ΔMC and ΔRV are not generally susceptible to detergents, as are *rfa* mutants. The resistance of ΔMC and ΔRV to EDTA, which is also an antibiotic to *rfa* strains, reinforces this distinction and indicates that expression of ΔMC and ΔRV does not grossly perturb the structure of the outer membrane (*14*).

To address the possibility that the mutant FepA proteins contained gross structural abnormalities that were responsible for their pore-forming phenotype, we evaluated their in vivo conformations. Binding experiments with monoclonal antibodies (MAbs) that recognize known epitopes of the receptor (*7*) indicated that the conformations of ΔMC and ΔRV were similar to that of native FepA (Table 2). Epitopes both upstream and downstream from central deletions were properly localized (either on the cell surface or buried in the bilayer) in the outer membranes of bacteria that expressed ΔMC and ΔRV. However, ΔMC and ΔRV differed from FepA in that several epitopes in the region of amino acids 100 to 142 (recognized by MAbs 5, 11, and 27), which are inaccessible on the surface of the wild-type receptor, were detected on the cell surface of bacteria that carried *fepA*ΔMC or *fepA*Δ*RV*. The simplest explanation for this result is that the epitopes in question are surface localized in native FepA but are sterically shielded by peptides that the *fepA*ΔMC and *fepA*Δ*RV* deletions eliminate (*15*). Aside from this alternation, the surface topologies of ΔMC, ΔRV, and ΔH261 were indistinguishable from that of FepA.

Given the likely existence of a channel in FepA, it was of interest to determine the

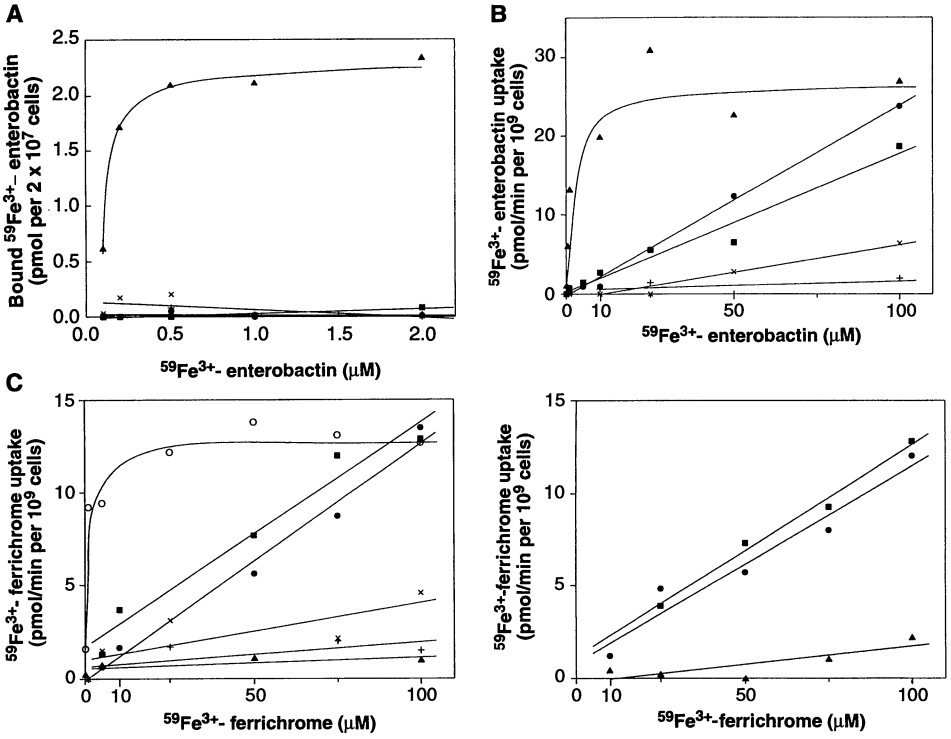

Fig. 3. Biochemical properties of FepA deletion mutants. Enterobactin was purified, complexed with $^{59}Fe^{3+}$ (*7*), and separated by chromatography on Sephadex LH20 (Pharmacia) (*18*). KDF541, either without plasmid or harboring *fepA*$^+$ (▲), *fepAΔH261* (+), *fepAΔRV* (■), *fepAΔMC* (●), or *fepAΔA306* (×) derivatives of pUC18 was grown for approximately 8 hours in MOPS medium (*23*) with appropriate antibiotics to the late exponential phase, collected by centrifugation, and washed with and suspended in MOPS medium. In each experiment, the expression of FepA and FepA mutants was quantitated with MAb 41 and ^{125}I–protein A as described in Figs. 1 and 2. Expression was relatively consistent, but as much as 20% variation in the amounts of FepA and the FepA mutants was observed from day to day. Expression of ΔA306 was always ~40% of FepA expression. Nonspecific background adsorption of siderophores to KDF541 was determined and subtracted to generate the plotted values. (**A**) Binding of ferric enterobactin. Bacteria were shaken for 2 hours at 37°C in MOPS medium without glucose so that their energy stores would be depleted. All subsequent steps were performed at 0°C (*24*). Cells were incubated for 30 min with $^{59}Fe^{3+}$-enterobactin at the indicated concentrations, separated by centrifugation, and washed. Bacteria-associated radioactivity was determined by scintillation spectroscopy. Data points are mean values from three experiments. (**B** and **C**) Uptake of siderophores by FepA deletion mutants. Initial rates of $^{59}Fe^{3+}$-enterobactin (B) and $^{59}Fe^{3+}$-ferrichrome (C) transport (*24*) were measured over the concentration range 0.1 to 100 μM. Duplicate experiments were performed with energy-depleted bacteria (*24*) at 0°C; all accumulation of $^{59}Fe^{3+}$ was energy dependent. Data points are mean values from at least four experiments; lines were drawn by Enzfitter version 1.0 (Elsevier). Wild-type FepA and FhuA, the ferrichrome receptor (*1*) [RWB18-60 (○) is the *fepA, fhuA*$^+$ parent of KDF541], showed Michaelis-Menten saturation kinetics for ferric enterobactin [Michaelis constant (K_m) = 0.2 μM; V_{max} = 25 pmol/min per 10^9 cells] and ferrichrome (K_m = 0.5 μM; V_{max} = 12 pmol/min per 10^9 cells) that were comparable to published values (*24, 25*). **Fig. 4 (lower right).** Uptake of ferrichrome in a *tonB* genetic background. The initial rates of $^{59}Fe^{3+}$-ferrichrome transport were determined as in Fig. 3; all uptake was energy dependent. (●) KDF571 (*tonB*) (*20*)/pΔMC (*fepAΔMC*); (■) KDF571/pΔRV (*fepAΔRV*); and (▲) KDF571/pITS449 (*fepA*$^+$) (*10*). Nonspecific background adsorption of siderophore to KDF571 was determined and subtracted from the plotted data, which are mean values from three experiments.

dependence of the pore on TonB function. Both siderophore uptake experiments and antibiotic sensitivity tests indicated that ΔMC and ΔRV promoted transport across the outer membrane independently of TonB (Fig. 4 and Table 1). Furthermore, *tonB* strains that expressed *fepAΔRV* or *fepAΔMC* efficiently accumulated ferrichrome in the cytoplasm in an energy-dependent manner, which confirms that TonB does not participate in the transport of molecules across the cytoplasmic membrane (*3*).

Because deletions that eliminate the ligand-binding residues of FepA circumvent the need for TonB, we propose that TonB normally acts to facilitate the movement of bound ferric enterobactin into the FepA channel. According to this view, TonB functions as a molecular gatekeeper in the bacterial cell envelope, which would be a protein that opens the FepA channel to ligands bound on the cell surface. This conclusion is consistent with existing data on TonB structure and function (*16*). The mechanism of TonB-dependent outer membrane transport therefore involves ligand binding to a surface domain of a receptor, followed by direct or indirect interaction of that protein with TonB, which facilitates movement of the ligand into an underlying channel and subsequently into the periplasm. Such a mechanism generally preserves the permeability barrier of the outer membrane but, for example, operates in response to the interaction of FepA with ferric enterobactin. The extensive sequence homology among the TonB-dependent proteins of *E. coli* (*3*) and other Gram-negative bacteria (*16, 17*) suggests that all ligand-specific outer membrane transport proteins may function by this gated-porin mechanism.

Table 1. Antibiotic sensitivity of *E. coli* K-12 strains that express *fepA* deletion mutations. Tests were performed as described (*13*); bacteria (*7, 13, 19, 20*) were plated on Luria broth agar, and disks that contained antibiotics or other compounds were applied to the agar. Susceptibility was evaluated after 12 hours and is expressed as the diameter in millimeters of the zone of growth inhibition. The experiment was performed three times with negligible variability (<5%); tabulated values are from a single experiment. The amounts of the various compounds tested and their molecular masses were as follows: SDS (750 μg; 288 daltons), EDTA (1.5 μg; 292 daltons), chloramphenicol (Cm; 30 μg; 323 daltons), deoxycholate (DEO; 750 μg; 392 daltons), gentamycin (Gm; 10 μg; 477 daltons), neomycin (Nm; 10 μg; 614 daltons), novobiocin (Nb; 30 μg; 634 daltons), erythromycin (Er; 15 μg; 734 daltons), rifampin (Ra; 5 μg; 823 daltons), and bacitracin (B; 30 μg; 1421 daltons).

Strain/plasmid	Relevant genotype	Compound susceptibility (mm)									
		SDS	EDTA	Cm	DEO	Gm	Nm	Nb	Er	Ra	B
		fepA *mutants*									
BN1071	*fepA*$^+$, *ompF*$^+$	0	0	22	0	16	10	10	0	11	0
KDF541	*fepA, ompF*$^+$	0	0	22	0	16	10	10	0	10	0
KDF541/pITS449	*fepA*$^+$, *ompF*$^+$	0	0	20	0	16	10	10	0	13	0
KDF541/pΔRV	*fepAΔRV, ompF*$^+$	15	0	28	0	16	11	16	15	19	12
KDF541/pΔMC	*fepAΔMC, ompF*$^+$	13	0	26	0	16	10	16	15	18	12
KDF541/pΔH261	*fepAΔH261, ompF*$^+$	0	0	22	0	16	10	10	0	12	0
KDF541/pΔA306	*fepAΔA306, ompF*$^+$	0	0	20	0	16	10	10	0	16	0
		tonB *mutants*									
KDF571	*fepA, tonB, ompF*$^+$	0	0	22	0	16	10	10	0	10	0
KDF571/pITS449	*fepA*$^+$, *tonB, ompF*$^+$	0	0	20	0	16	10	10	0	13	0
KDF571/pΔMC	*fepAΔMC, tonB, ompF*$^+$	15	0	28	0	16	12	16	15	20	13
		ompF *mutants*									
PLB3261	*fepA*$^+$, *ompF*$^+$	0	0	20	0	16	10	0	0	8	0
PLB3268	*fepA*$^+$, *ompFΔ114–120*	16	0	21	14	16	10	15	15	20	17
OC103	*fepA*$^+$, *ompFΔ118–133*	15	0	22	13	16	10	17	13	20	16
OC110	*fepA*$^+$, *ompFΔ111–123*	16	0	22	14	16	10	18	15	21	16

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1. Examples include the recognition of ferric enterobactin and colicins B and D by FepA [S. Guterman, *J. Bacteriol.* **114**, 1217 (1973); A. P. Pugsley and P. Reeves, *ibid.* **126**, 1052 (1976); R. R. Wayne, K. Frick, J. B. Neilands, *ibid.*, p 7]; the recognition of vitamin B_{12}, bacteriophage BF23, and colicins E1 and E3 by BtuB [D. R. DiMasi *et al.*, *ibid.* **115**, 56 (1973)], and the recognition of ferrichrome, colicin M, albomycin, and bacteriophages T1, T5, and ϕ80 by TonA (FhuA) (*18*). See also J B. Neilands, *Annu. Rev. Microbiol.* **36**, 285 (1982); B. Lugtenberg and L. van Alphen, *Biochim. Biophys. Acta* **737**, 51 (1981).
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Table 2. Cytofluorimetric localization of surface and buried epitopes of FepA. The deep rough *E. coli* K-12 strain KDF669 (*rfa::Tn5, fepA*), either without plasmid or carrying pITS449, pΔH261, pΔMC, pΔRV, or pΔA306, was grown to late log phase (~6 hours) in MOPS medium, stained with MAb to FepA and fluorescein isothiocyanate–labeled goat antibodies to mouse immunoglobulin (*7*), and analyzed on an EPICS Profile II flow cytometer (Coulter). Values in the table are mean fluorescence intensity. Samples were considered positive if greater than 70% of the bacterial population showed a mean fluorescence intensity (range 0 to 255) that was more than twice the background level established by KDF669 (52). Bold values indicate that greater than 95% of the cell population was fluorescent (*21*). The experiment was performed three times with negligible variability (<5%). Negative samples are designated with a minus.

	Mean fluorescence intensity																				
	Buried epitopes									Surface epitopes											
FepA residues:	100–142a					100–142b		444–475	495–566	1–24	200–227		258–290		314–339				382–400		
MAb:	5	7	11	27	38	2	3	57	64	29	33	34	16	44	31	35	37	45	23	24	
Strain/plasmid																					
KDF669	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
KDF669/pITS449	–	–	–	–	–	–	–	–	–	152	**210**	**183**	**169**	**198**	**207**	**208**	**187**	**210**	**206**	**201**	
KDF669/pΔH261	–	–	–	–	–	–	–	–	–	125	**223**	**228**	**198**	**226**	**216**	**207**	**203**	**234**	**220**	**225**	
KDF669/pΔMC	**218**	–	**187**	110	–	–	–	–	–	150	–	–	–	–	–	–	–	–	**229**	**231**	
KDF669/pΔRV	**141**	–	**187**	120	–	–	–	–	–	129	–	–	–	–	–	–	–	–	**167**	**206**	
KDF669/pΔA306	–	–	–	–	–	–	–	–	–	140	–	127	–	–	–	–	–	–	–	–	

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12. ΔA306 showed enhanced sensitivity to rifampin as well as some uptake of siderophores at high concentrations, which suggests that the *fepAΔA306* mutation may also expose the FepA pore although to a lesser extent than *fepAΔMC* and *fepAΔRV*.
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14. If the expression of ΔMC and ΔRV caused a generalized, nonspecific increase in outer membrane permeability, then we would expect them to confer increased susceptibility to the aminoglycosides gentamycin and neomycin, which was not observed.
15. We cannot exclude the alternative explanation that the deletions alter the thermodynamic equilibrium between the receptor and its membrane environment, inducing NH_2-terminal conformational changes that accommodate this perturbation. The fact that the NH_2-terminal topology of ΔA306 is disrupted by the COOH-terminal mutation (the majority of the epitopes in the receptor's ligand-binding domain are inaccessible to MAb recognition) may support this view. The gross structural changes seen in ΔA306 probably explain why it does not bind ferric enterobactin.
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19. KDF541 was derived from RWB18-60 (*pro, leu, trp, entA, ΔrecA, ΔfepA*) (*10*) by sequential selection for resistance to colicin Ia (*cir*) and bacteriophage T5 (*fhuA*). Hybridization analysis of RWB18-60 shows no evidence of *fepA* sequence (M. A. McIntosh, unpublished data).
20. We created KDF571 by selecting for simultaneous resistance of RWB18-60 to colicins Ia and M. Putative *tonB* mutants were transformed with pRZ540 [*tonB*$^+$; K. Postle and W. S. Reznikoff, *J. Mol. Biol.* **131**, 619 (1979)] and tested for sensitivity to colicins Ia and M and uptake of ferrichrome (*18*). To generate *cir* and *fhuA* markers, which were confirmed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) of mutant cell envelopes, we sequentially selected the *tonB*/pRZ540 strains for resistance to colicin Ia and bacteriophage T5. The resultant strain, KDF570/pRZ540 (*pro, leu, trp, B1, entA, ΔrecA, ΔfepA, cir, fhuA, tonB/tonB*$^+$) was cured of pRZ540 with acridine orange [J. H. Miller, Ed., *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1978), p. 104] and transformed with the desired pUC18 derivatives.
21. MAb 29 binding to bacteria that expressed FepA was not seen in other studies (*7*). However, in this study we used KDF669, a nonreverting *rfa*::transposon *5* (Tn*5*) mutant that produces a deep rough lipopolysaccharide core, as host for the various plasmids. In this background, *fepA*$^+$ cells were weakly fluorescent, which indicates that the epitope recognized by MAb 29 is accessible on the cell surface but lies deep within the core sugars.
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DNA Polymerase β and DNA Synthesis in *Xenopus* Oocytes and in a Nuclear Extract

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The identities of the DNA polymerases required for conversion of single-strand (ss) M13 DNA to double-strand (ds) M13 DNA were examined both in injected *Xenopus laevis* oocytes and in an oocyte nuclear extract. Inhibitors and antibodies specific to DNA polymerases α and β were used. In nuclear extracts, inhibition by the antibody to polymerase β could be reversed by purified polymerase β. The polymerase β inhibitors, dideoxythymidine triphosphate (ddTTP) and dideoxycytidine triphosphate (ddCTP), also blocked DNA synthesis and indicated that polymerase β is involved in the conversion of ssDNA to dsDNA. These results also may have particular significance for emerging evidence of an ssDNA replication mode in eukaryotic cells.

Xenopus laevis oocytes and eggs have been used to study eukaryotic transcription, translation, intracellular transport and localization of molecules, DNA replication (*1*), and DNA repair (*2, 3*). We used *Xenopus* oocytes to study the mechanisms of DNA replication. An extensive component of genomic DNA replication in *Xenopus* embryos appears to be conversion of long segments of ssDNA to semiconservatively replicated dsDNA molecules (*4*). Thus, we used ssM13 DNA molecules as a model for conversion of ssDNA to dsDNA (*5*) in oocytes and in an oocyte nuclear extract. This DNA synthesis appears to be dependent on the activity of DNA polymerase β and the activity of DNA polymerase α; DNA polymerase δ or ε or both may also be required. Polymerase β is a highly conserved DNA polymerase in vertebrates (*6*), generally considered to have a role in gap-filling DNA synthesis in DNA repair (*7*). Recently, polymerase β was shown to substitute for DNA polymerase I in the joining of Okazaki fragments during DNA replication in *Escherichia coli* (*8*).

Model DNA replication systems that use small ds viral DNA molecules (*9*) do not appear to depend on DNA polymerase β because the systems can be reconstituted with purified proteins in the absence of DNA polymerase β. Other replication models (*10*), however, are important to

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