SepL Resembles an Aberrant Effector in Binding to a Class 1 Type III Secretion Chaperone and Carrying an N-Terminal Secretion Signal^{†‡}

Rasha Younis,^{1†} Lewis E. H. Bingle,^{1†} Sarah Rollauer,² Diana Munera,³ Stephen J. Busby,¹ Steven Johnson,² Janet E. Deane,^{2§} Susan M. Lea,² Gad Frankel,³ and Mark J. Pallen^{1*}

School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom¹; Sir William Dunn School of Pathology, University of Oxford, South Parks Rd., Oxford OX1 3RE, United Kingdom²; and Centre for Molecular Microbiology and Infection, Division of Cell and Molecular Biology, Imperial College London, London SW7 2AZ, United Kingdom³

Received 30 June 2010/Accepted 31 August 2010

Here we show that the type III secretion gatekeeper protein SepL resembles an aberrant effector protein in binding to a class 1 type III secretion chaperone (Orf12, here renamed CesL). We also show that short N-terminal fragments (≤70 amino acids) from SepL are capable of targeting fusion proteins for secretion and translocation.

Strains of enterohemorrhagic and enteropathogenic *Escherichia coli* (EPEC) exploit a type III secretion system (T3SS) encoded by the locus for enterocyte effacement (LEE) to mediate interactions with intestinal cells (10, 24). Two chaperones that bind effectors (type I chaperones) have been identified within this system: CesT and CesF (7, 8). Gene expression within this system is dependent on the transcriptional activator Ler (9). The activity of Ler is modulated by, among other things, a poorly characterized 117-amino-acid LEE-encoded protein known as Orf12, ST22, or Mpc (27). The specificity of protein secretion with the LEE-encoded system depends on the protein SepL and its partner, SepD (6, 16, 20). The prevailing hypothesis is that these proteins initially engage with

the secretion apparatus to form a gate permissive to translocator export but hindering effector secretion and that this gate opens when bacteria interact with host cells (5, 6).

Sequence analyses have shown that SepL is a member of the YopN-TyeA family of proteins (21, 22). Some other members of this family resemble aberrant effectors in that they bind to class 1 chaperones (22, 26), undergo secretion and translocation, or produce effects within eukaryotic cells (1, 4, 12, 13, 15, 18). This led us to suspect that SepL might also bind to one or more class 1 chaperones and might also carry a secretion signal. We looked for homologues of the YopN chaperone, SycN, using PSI-BLAST to search the nonredundant protein database. By the third iteration, a significant hit to EsaM from

FIG. 1. Multiple alignment of CesL with SycN, EsaM, and SsaM. Multiple alignment performed using ClustalW is shown, with dark shading indicating positions where sequences are identical and light shading indicating positions that show conservative changes. The secondary structure of SycN is shown beneath the alignment. H, helix; E, sheet.

* Corresponding author. Mailing address: School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom. Phone: 44 121 414 7163. Fax: 44 121 414 5925. E-mail: m.pallen@bham.ac.uk.

† These authors contributed equally to this work.

§ Present address: CIMR Haematology, University of Cambridge, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, United Kingdom.

‡ Supplemental material for this article may be found at <http://j.b.asm.org/>.

Published ahead of print on 10 September 2010.

SpiC	1	-MSEEGFMIAVLKGIPLDODIRAEG-NSRSWIMTIDG--HPARGEISEAFSISLFTN-DLESIPKPCIA
SG1281	1	-----MNKFRSVPLFNIELSE-KGDMVIMSIDN--LPAQVYVSTLSEYFTILLD-DTKNLSDTSMH
CV_2598	1	-----MKLPDSIAALEWVCVLSPRSDRWLLDIAG--VPAWGWSYPQGITTAEI--ELEVVDDAWIQ
CATC2_22927	1	-----MSAWFGIEDIYWEKKSHAGRSVFSVEVGFLPELTIEDMHFWLLVQIPKPKDKNIELVWIK
SepD	1	MNNNGIAKNDCDWLTALDFVKDVNGSPTLTFTYIYQ--KNAFLHDEGNYWVLYTEISGDFRQIPTDTFI
SpiC	66	YVTLALLAHPVVDYATOLTADGWLNGYVTTSSSELIAIETEKHLATCTILKNVIR-----NHHKLYS
SG1281	60	YVTLALLAHPVVDYAVOLTAAGIWLCVYDELLTNEALATELEKQALARYTNVVASICTISQEARHE
CV_2598	59	RVCLWLASIRTNLDDGLLERGKLYVRRHEHGLSAVEWEATLNQOLAVVAMLVKHAASSISSIGSAAGR
CATC2_22927	63	YTGILLAN-NANYGVFLORDNTWLVRRYTASCSMEITQRSTSEHTATVIGRRVORLIQSGIRGDA
SepD	69	RICNLLAVSKYKQMGIFLSNKKWYLCQIETHKDNHRANMSKAIMCHTLASLIDKQFDKEQSSSDTMM
SpiC	131	GGV-----
SG1281	130	VEL-----
CV_2598	129	V-----
CATC2_22927	132	EISDKPLFHTTINGWV
SepD	139	PPTHLFSDIGRIV---

FIG. 2. Multiple alignment of SepD, SpiC, and selected homologues. Multiple alignment performed using ClustalW is shown, with dark shading indicating positions where sequences are identical and light shading indicating positions that show conservative changes. Species of origin and accession numbers: SpiC, *Salmonella enterica*, Swissprot accession number P74863.1; SG1281, *Sodalis glossinidius*, GenBank accession number BAE74556.1; CV_2598, *Chromobacterium violaceum*, GenBank accession number AAQ60268.1; CATC2_22927, *Citrobacter youngae*, NCBIREF-SEQ accession number ZP_06356284; SepD, *Escherichia coli*, GenBank accession number CAA90273.1.

Edwardsiella tarda (expect value, $3e-21$; 22% identity over 117 residues), which is a homologue of SsaM from the *Salmonella* pathogenicity island 2 (Spi-2) system, was reported. A subsequent PSI-BLAST search with EsaM returned, within three iterations, significant hits (expect value, $<4e-21$; 17% identity over 117 residues) to Orf12. Although PSI-BLAST searches with Orf12 failed to return significant hits to SycN, four Orf12

homologues returned significant hits (expect value, $<10^5$) to the type III SycN domain (TIGR02503) when searched against the Conserved Domain Database (data not shown). From these results, we concluded that Orf12 is a homologue of SycN and can be classified as a class 1 chaperone. Given the interaction between SycN and YopN, we hypothesized that Orf12 interacts with SepL and renamed this protein CesL, in line with

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description ^a	Source or reference
Strains		
DH5 α derivative NEB 5-alpha	<i>fhuA2</i> Δ (<i>lacZ-argF</i>)U169 <i>phoA</i> <i>glnV44</i> ϕ 80d <i>lacZ</i> Δ M15 <i>gyrA96</i> <i>recA1</i> <i>relA1</i> <i>endA1</i> <i>thi-1</i> <i>hsdR1</i> ; cloning host for pulldown constructs	New England BioLabs
TUV 93-0	Stx ⁻ derivative of enterohemorrhagic <i>E. coli</i> strain EDL933	Arthur Donohue-Rolfe (Tufts Cummings School of Veterinary Medicine)
B834(DE3)	Cloning host for studies on protein complexes	Merck
E2348/69	Wild-type EPEC O127:H6	19
E2348/69 Δ <i>escN</i>		14
E2348/69 Δ <i>sepD</i>		20
E2348/69 Δ <i>sepL</i>		20
Plasmids		
pSA10	<i>lacI</i> ^q / <i>tacp</i> expression vector (Ap ^r)	25
pRYC2casA	<i>lacI</i> ^q / <i>tacp</i> expression vector (Ap ^r)	This study
pRY9	<i>lacI</i> ^q / <i>tacp-cesL</i> expression vector (Ap ^r)	This study
pRY19	<i>lacI</i> ^q / <i>tacp-3</i> $\times$ <i>FT-cesL</i> expression vector (Ap ^r)	This study
pET-Duet, pACYC-Duet, pCOLA-Duet	Expression vectors for studies on protein complexes	Merck
pSepDL	pET-Duet expressing His-tagged SepL from insert in MCS1 and untagged SepD from insert in MCS2	
pCesL	pET-Duet expressing His-tagged CesL from insert in MCS1	
pSepD	pACYC-Duet expressing untagged SepD from insert in MCS2	
pSepL	pCOLA-Duet expressing untagged SepL from insert in MCS2	
pCX340	pBR322 derivative; cloning vector used to fuse genes to <i>blaM</i> , which encodes the mature form of TEM-1 β -lactamase	3
pICC550	Residues 1–20 of SepL fused to TEM-1	This study
pICC551	Residues 1–50 of SepL fused to TEM-1	This study
pICC552	Residues 1–70 of SepL fused to TEM-1	This study
pICC553	Residues 1–170 of SepL fused to TEM-1	This study
pICC554	Residues 1–267 of SepL fused to TEM-1	This study
pICC555	Residues 1–351 of SepL fused to TEM-1	This study

^a MCS1, multiple-cloning site 1.

TABLE 2. Primers used in this study

Primer	Sequence
TOPO-3XFT-NT-CesL-F ^a	CAAAATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGAC ATCGACTACAAAGACGATGACGACAAGGGAATGAATCTTTTAGTT AAAAGAAACG
CesL-R	TTATCATGATGTTCATCCTGCGAACG
For-20SepL	TATGGCTAATGGTATTGAATTTAATCAAAACCCCGCATCTGTTTTTA ATTCTAATTCATTAGAG
Rev-20SepL	AATTCTCTAATGAATTAGAAATTAACAGATGCGGGGTTTTGATT AAATTCATACCATTAGCCA
NdeI-SepL	GGAATAACATATGGCTAATGGTATTGAATTTA
50SepL-EcoRI	GTGCGAATTCTCCATTGCTAATTCATTTTGCAAAT
70SepL-EcoRI	GTGCGAATTCTCACCTTTGCGATATCCCAGGCTTA
170SepL-EcoRI	GTGCGAATTCTCCAGGAAAGCAGAGCCAGCTCCC
267SepL-EcoRI	GTGCGAATTCTCGATTGCAATAATATCCGTATAGT
SepL-EcoRI	GTGCGAATTCTCCATAACATCCTCCTTATAATCTA

^a Used to add the triple FLAG tag epitope (3XFT) to the CesL protein to enable immunoaffinity pulldown.

existing conventions. A multiple alignment of SycN and Orf12 homologues highlights several conserved residues from the hydrophobic core of this class of chaperone (Fig. 1).

SepL binds to the protein SepD, while SsaL, the homologue of SepL in the Spi-2 system, is a component of the SsaM/SsaL/SpiC complex (29). A PSI-BLAST search with SpiC found unequivocal homologues in all the Esc-Ssa T3SSs and returned a hit to SepD within 5 iterations, albeit with unimpressive statistics (E value, 6.8; 13% identity; 13% over 111 residues). A multiple alignment of SepD with SpiC and its homologues highlighted several conserved patches of sequence (Fig. 2). On the basis of the sequence analyses, we concluded that SepD is almost certainly a homologue of SpiC and that the triplet of proteins CesL, SepL, and SepD is homologous to the SsaM/SsaL/SpiC complex from the Spi-2 system (29).

To confirm our prediction that CesL interacts with the SepD/SepL complex, triple-Flag-tagged CesL was expressed under LEE-inducing conditions in enterohemorrhagic *E. coli* (Tables 1 and 2) and then subjected to rapid immunoaffinity pulldown, using an established protocol (17, 28). Seven protein bands were visualized with Coomassie staining after polyacrylamide gel electrophoresis (Fig. 3). Peptide mass fingerprinting of gel slices assigned protein identities with significant *P* values to the bands as follows: band 1, AceE and Ler; band 2, DnaK; band 3, GroEL; band 4, SepL; band 5, SepL; band 6, 50S ribosomal protein L1; and band 7, Ler, CesL, and SepD. This analysis confirmed that a complex containing SepL and SepD could be pulled down by CesL. In addition, this assay confirmed the known interaction between CesL and Ler (27).

In otherwise identical pulldown experiments in which the Flag-tagged bait protein was Ler, the only T3SS-associated protein identified by Fourier transform ion cyclotron resonance (FT-ICR) analysis of bands visible with Coomassie staining was Ler itself, which, as in the CesL pulldown assay (Fig. 3), appeared to form high-molecular-weight sodium dodecyl sulfate (SDS)-resistant multimeric complexes (see the figure in the supplemental material). This excludes the possibility that SepL and SepD interact nonspecifically with the Flag tag or with the antibody to it.

The fact that the chaperone CesL can bind to Ler, as well as to SepD/SepL, suggests that this protein interaction network might couple protein secretion to regulation of gene expres-

sion in the LEE-encoded system, mirroring similar regulatory networks seen in other type III secretion systems (2).

One of the hallmarks of type III secretion chaperones is that they enhance stability and/or prevent aggregation of their substrates within the *E. coli* cytoplasm (11, 23). The difficulties of creating a nonpolar *cesL* mutant have already been documented (27). Therefore, rather than rely on a mutagenesis strategy, various combinations of His-tagged and untagged proteins from the potential CesL/SepD/SepL complex were expressed in a laboratory strain of *E. coli* (Table 1) and purified by Ni-nitrilotriacetic acid (NTA) affinity chromatography. Cells were grown in LB buffer with appropriate antibiotics to an optical density at 600 nm (OD₆₀₀) of 0.6 and induced with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) at 20°C for

FIG. 3. Immunoaffinity pulldown assay with Flag-tagged CesL. Sizes of molecular weight marker bands (Novex Sharp prestained protein standard; Invitrogen) are indicated on the left. Immunoaffinity-isolated protein bands with identities assigned by FT-ICR mass spectroscopy are shown on the right. Predicted molecular weights: Ler, 14.3; Flag-tagged CesL, 16.6; SepD, 17.6; SepL, 40.

FIG. 4. SepL complexes following Ni-NTA purification and size exclusion chromatography. Lane 1, His-tagged SepL and untagged SepD (expressed from pSepDL); lane 2, His-tagged SepL (expressed from pSepL); lane 3, His-tagged CesL and untagged SepD and SepL (expressed from pCesL, pSepD, and pSepL, respectively); lane M, molecular weight markers.

16 h. After centrifugation (30 min, 4,500 rpm, 4°C), cell pellets were frozen at -20°C and then resuspended in lysis buffer (20 mM Tris [pH 8.0], 500 mM NaCl, 20 mM imidazole, and complete EDTA-free protease inhibitor tablets, [Roche]) and lysed using an EmuFlex-C5 homogenizer (Avestin). The resulting cell suspension was centrifuged (30 min, 20,000 rpm, 4°C) and applied to a 5-ml His trap Ni-NTA column (Qiagen) washed in lysis buffer. Protein was eluted using a gradient of 0 to 1 M imidazole in 20 mM Tris (pH 8.0)–150 mM NaCl. Fractions containing the complexes were further purified by size exclusion chromatography, using a HiLoad 26/60 Superdex 200 column preequilibrated in running buffer (20 mM Tris [pH 8.0]–150 mM NaCl). Fractions were pooled and concentrated to 16 mg ml^{-1} , using Millipore Ultra-15 centrifugal filtration devices with a cutoff of 10 kDa, and stored at 4°C .

Of the combinations tried, only His-tagged SepL alone, His-tagged SepL/untagged SepD, and His-tagged CesL/untagged SepD/untagged SepL produced soluble protein complexes (Fig. 4). The protein samples were further purified using an

additional round of size exclusion chromatography and were subjected to multiangle light scattering (MALS) to assess the stoichiometry of the complexes. This round of size exclusion chromatography was performed on a Superdex 200 10/300 column (GE Healthcare) equilibrated in 25 mM Tris-HCl [pH 7.5]–150 mM NaCl at 0.4 ml/min. The column was followed in-line by a Dawn Heleos II light-scattering detector (Wyatt Technologies) and an Optilab Rex refractive index monitor (Wyatt Technologies). Molecular mass calculations were performed using ASTRA 5.3.4.14 (Wyatt Technologies), assuming a refractive index increment (dn/dc value) of 0.186 ml/g .

SepD/SepL demonstrated concentration-dependent aggregation, which manifested as a shift in the elution position off the column toward a higher molecular weight, coupled to an increased calculated mass at the center of the elution peak (Fig. 4). Calculated molecular weight values ranged from 71 at 0.5 mg/ml to 105 at 16 mg/ml . Two representative traces are shown in Fig. 5. The CesL/SepD/SepL complex, on the other hand, consistently eluted at the same point regardless of concentration, and the calculated molecular weight values ranged from 73 at 0.75 mg/ml to 76 at 12 mg/ml . These are within the experimental error of the technique and are in good agreement with the calculated mass of a 1:1:1 complex ($15.5\text{ kDa} + 17\text{ kDa} + 42\text{ kDa}$).

These results suggest that the heterotrimeric CesL/SepD/SepL complex resembles the SycN/YscB/YopN complex, although it remains uncertain whether SepD is a homologue of YscB (an assignment supported by the conserved position of the *sepD* and *yscB* genes, adjacent to *escC* and *yscC*, respectively); only structural studies will resolve this issue.

Next, we investigated whether SepL carried an N-terminal secretion signal by creating a series of constructs (Tables 1 and 2) expressing progressively longer N-terminal portions of SepL fused to a β -lactamase reporter, TEM-1 (3). Under LEE-inducing conditions, three short N-terminal fusions (20, 50, and 70 amino acids from SepL [20SepL, 50SepL, and 70SepL,

FIG. 5. Analysis of SepD/SepL and CesL/SepD/SepL complexes by size exclusion chromatography followed by in-line multiangle light scattering. The left axis represents the molecular mass at any given point in the elution profile. Elution profiles of SepD/SepL complexes are shown at 2 mg/ml and 8 mg/ml and demonstrate concentration-dependent increases in the masses of the peaks. Elution profiles of CesL/SepD/SepL complexes are shown at 1.5 mg/ml and 6 mg/ml and demonstrate a stable, concentration-independent 1:1:1 complex.

FIG. 6. Secretion and translocation of SepL N-terminal fragments fused to TEM. (a) Secretion profiles reveal that 20SepL, 50SepL, and 70SepL but not 170SepL, 267SepL, or full-length SepL can mediate TEM secretion from wild-type EPEC and the *sepD* and *sepL* mutants. Neither protein was secreted from the *escN* mutant. (b) Analysis of whole-cell lysates reveals comparable expression levels of the SepL-TEM fusions in the different EPEC strains. Molecular mass markers (in kDa) are indicated on the left. (c) 20SepL, 50SepL, and 70SepL but not 170SepL, 267SepL, or full-length SepL mediate TEM protein translocation specifically from wild-type EPEC. The presented data are averages of triplicate values of results obtained from two independent experiments.

respectively)) were secreted from wild-type cells and from *sepD* and *sepL* mutants but not from cells defective in type III secretion (Fig. 6a). Three longer fusions encoding 170 [170SepL], 267 [267SepL], and 351 (full-length) amino acids from SepL were not secreted from any strain (Fig. 6a). Immunoblots of whole-cell lysates revealed equal expression of TEM fusion proteins in the different EPEC strains (Fig. 6b). Translocation assays showed that the three shorter fusions were translocated into HeLa cells, although the efficiency of translocation declined with length. No translocation could be detected for the three longer fusions (Fig. 6c). Our favored interpretation of these results is that the central and C-terminal portions of SepL hinder the protein's secretion and translocation under nonpermissive conditions and have to undergo a conformational change before secretion can occur and that this conformational change is disrupted by large C-terminal fusions. However, further experiments are required to establish the physiological significance of SepL's N-terminal secretion signal.

In conclusion, we established that SepL, like several of its homologues, resembles a T3SS effector in carrying secretion signals and binding to a class 1 chaperone, and we identified a novel protein interaction network linking a regulator of protein secretion (SepL) to a regulator of gene expression (Ler).

We thank David Lee for advice on rapid immunoaffinity pulldown and Cleidiane Zamprorio for performing and advising on FT-ICR MS analysis.

Rasha Younis and Lewis E. H. Bingle were supported by BBSRC grant BBE0208601.

REFERENCES

- Botteaux, A., M. P. Sory, L. Biskri, C. Parsot, and A. Allaoui. 2009. MxiC is secreted by and controls the substrate specificity of the *Shigella flexneri* type III secretion apparatus. *Mol. Microbiol.* **71**:449–460.
- Brutinel, E. D., and T. L. Yahr. 2008. Control of gene expression by type III secretory activity. *Curr. Opin. Microbiol.* **11**:128–133.
- Charpentier, X., and E. Oswald. 2004. Identification of the secretion and translocation domain of the enteropathogenic and enterohemorrhagic *Escherichia coli* effector Cif, using TEM-1 beta-lactamase as a new fluorescence-based reporter. *J. Bacteriol.* **186**:5486–5495.
- Day, J. B., F. Ferracci, and G. V. Plano. 2003. Translocation of YopE and YopN into eukaryotic cells by *Yersinia pestis* yopN, *tyeA*, *syncN*, *yscB* and *lcrG* deletion mutants measured using a phosphorylatable peptide tag and phosphospecific antibodies. *Mol. Microbiol.* **47**:807–823.
- Deane, J. E., P. Abrusci, S. Johnson, and S. M. Lea. 2010. Timing is everything: the regulation of type III secretion. *Cell. Mol. Life Sci.* **67**:1065–1075.
- Deng, W., Y. Li, P. R. Hardwidge, E. A. Frey, R. A. Pfuetzner, S. Lee, S. Gruenheid, N. C. Strynacka, J. L. Puente, and B. B. Finlay. 2005. Regulation of type III secretion hierarchy of translocators and effectors in attaching and effacing bacterial pathogens. *Infect. Immun.* **73**:2135–2146.
- Elliott, S. J., S. W. Hutcheson, M. S. Dubois, J. L. Mellies, L. A. Wainwright, M. Batchelor, G. Frankel, S. Knutton, and J. B. Kaper. 1999. Identification of CesT, a chaperone for the type III secretion of Tir in enteropathogenic *Escherichia coli*. *Mol. Microbiol.* **33**:1176–1189.
- Elliott, S. J., C. B. O'Connell, A. Koutsouris, C. Brinkley, M. S. Donnenberg, G. Hecht, and J. B. Kaper. 2002. A gene from the locus of enterocyte effacement that is required for enteropathogenic *Escherichia coli* to increase tight-junction permeability encodes a chaperone for EspF. *Infect. Immun.* **70**:2271–2277.
- Elliott, S. J., V. Sperandio, J. A. Giron, S. Shin, J. L. Mellies, L. Wainwright, S. W. Hutcheson, T. K. McDaniel, and J. B. Kaper. 2000. The locus of enterocyte effacement (LEE)-encoded regulator controls expression of both LEE- and non-LEE-encoded virulence factors in enteropathogenic and enterohemorrhagic *Escherichia coli*. *Infect. Immun.* **68**:6115–6126.
- Elliott, S. J., L. A. Wainwright, T. K. McDaniel, K. G. Jarvis, Y. K. Deng, L. C. Lai, B. P. McNamara, M. S. Donnenberg, and J. B. Kaper. 1998. The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic *Escherichia coli* E2348/69. *Mol. Microbiol.* **28**:1–4.
- Feldman, M. F., and G. R. Cornelis. 2003. The multitalented type III chaperones: all you can do with 15 kDa. *FEMS Microbiol. Lett.* **219**:151–158.
- Fu, Z. Q., M. Guo, and J. R. Alfano. 2006. *Pseudomonas syringae* HrpJ is a type III secreted protein that is required for plant pathogenesis, injection of effectors, and secretion of the HrpZ1 Harpin. *J. Bacteriol.* **188**:6060–6069.
- Garcia, J. T., F. Ferracci, M. W. Jackson, S. S. Joseph, I. Pattis, L. R. Plano, W. Fischer, and G. V. Plano. 2006. Measurement of effector protein injection by type III and type IV secretion systems by using a 13-residue phosphorylatable glycogen synthase kinase tag. *Infect. Immun.* **74**:5645–5657.
- Garmendia, J., A. D. Phillips, M. F. Carlier, Y. Chong, S. Schuller, O. Marches, S. Dahan, E. Oswald, R. K. Shaw, S. Knutton, and G. Frankel. 2004. TccP is an enterohemorrhagic *Escherichia coli* O157:H7 type III effector protein that couples Tir to the actin-cytoskeleton. *Cell. Microbiol.* **6**:1167–1183.
- Ho, T. D., and M. N. Starnbach. 2005. The *Salmonella enterica* serovar Typhimurium-encoded type III secretion systems can translocate *Chlamydia trachomatis* proteins into the cytosol of host cells. *Infect. Immun.* **73**:905–911.
- Kresse, A. U., F. Beltrametti, A. Muller, F. Ebel, and C. A. Guzman. 2000. Characterization of SepL of enterohemorrhagic *Escherichia coli*. *J. Bacteriol.* **182**:6490–6498.
- Lee, D. J., S. J. Busby, L. F. Westblade, and B. T. Chait. 2008. Affinity isolation and I-DIRT mass spectrometric analysis of the *Escherichia coli* O157:H7 Sakai RNA polymerase complex. *J. Bacteriol.* **190**:1284–1289.
- Lee, V. T., D. M. Anderson, and O. Schneewind. 1998. Targeting of *Yersinia* Yop proteins into the cytosol of HeLa cells: one-step translocation of YopE across bacterial and eukaryotic membranes is dependent on SycE chaperone. *Mol. Microbiol.* **28**:593–601.
- Levine, M. M., E. J. Bergquist, D. R. Nalin, D. H. Waterman, R. B. Hornick, C. R. Young, and S. Sotman. 1978. *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are non-invasive. *Lancet* **i**:1119–1122.
- O'Connell, C. B., E. A. Creasey, S. Knutton, S. Elliott, L. J. Crowther, W. Luo, M. J. Albert, J. B. Kaper, G. Frankel, and M. S. Donnenberg. 2004. SepL, a protein required for enteropathogenic *Escherichia coli* type III translocation, interacts with secretion component SepD. *Mol. Microbiol.* **52**:1613–1625.
- Pallen, M. J., S. A. Beatson, and C. M. Bailey. 2005. Bioinformatics analysis

- of the locus for enterocyte effacement provides novel insights into type-III secretion. *BMC Microbiol.* **5**:9.
22. **Pallen, M. J., S. A. Beatson, and C. M. Bailey.** 2005. Bioinformatics, genomics and evolution of non-flagellar type-III secretion systems: a Darwinian perspective. *FEMS Microbiol. Rev.* **29**:201–229.
23. **Parsot, C., C. Hamiaux, and A. L. Page.** 2003. The various and varying roles of specific chaperones in type III secretion systems. *Curr. Opin. Microbiol.* **6**:7–14.
24. **Perna, N. T., G. F. Mayhew, G. Posfai, S. Elliott, M. S. Sonnenberg, J. B. Kaper, and F. R. Blattner.** 1998. Molecular evolution of a pathogenicity island from enterohemorrhagic *Escherichia coli* O157:H7. *Infect. Immun.* **66**:3810–3817.
25. **Schlosser-Silverman, E., M. Elgrably-Weiss, I. Rosenshine, R. Kohen, and S. Altuvia.** 2000. Characterization of *Escherichia coli* DNA lesions generated within J774 macrophages. *J. Bacteriol.* **182**:5225–5230.
26. **Schubot, F. D., M. W. Jackson, K. J. Penrose, S. Cherry, J. E. Tropea, G. V. Plano, and D. S. Waugh.** 2005. Three-dimensional structure of a macromolecular assembly that regulates type III secretion in *Yersinia pestis*. *J. Mol. Biol.* **346**:1147–1161.
27. **Tsai, N. P., Y. C. Wu, J. W. Chen, C. F. Wu, C. M. Tzeng, and W. J. Syu.** 2006. Multiple functions of I0036 in the regulation of the pathogenicity island of enterohaemorrhagic *Escherichia coli* O157:H7. *Biochem. J.* **393**:591–599.
28. **Westblade, L. F., L. Minakhin, K. Kuznedelov, A. J. Tackett, E. J. Chang, R. A. Mooney, I. Vvedenskaya, Q. J. Wang, D. Fenyo, M. P. Rout, R. Landick, B. T. Chait, K. Severinov, and S. A. Darst.** 2008. Rapid isolation and identification of bacteriophage T4-encoded modifications of *Escherichia coli* RNA polymerase: a generic method to study bacteriophage/host interactions. *J. Proteome Res.* **7**:1244–1250.
29. **Yu, X. J., K. McGourty, M. Liu, K. E. Unsworth, and D. W. Holden.** 2010. pH sensing by intracellular *Salmonella* induces effector translocation. *Science* **328**:1040–1043.