Supermolecular structure of the enteropathogenic Enterobacteriaceae coli type III secretion system and its direct interaction with the EspA-sheath-like structure

Kachiko Sekiya*†, Minako Ohishi*†§‡, Tomoaki Ogino§*, Koichi Tamano*, Chihiro Sasakawa†, and Akio Abe*§**

*Laboratory of Electron Microscopy, School of Pharmaceutical Sciences, and †Laboratory of Bacterial Infection, Kitasato Institute for Life Sciences, Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan; ‡Laboratory of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan; and §Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Communicated by Satoshi Omura, The Kitasato Institute, Tokyo, Japan, July 23, 2001 (received for review June 20, 2001)

Enteropathogenic Enterobacteriaceae coli (EPEC) secretes several Esp proteins via the type III secretion system (secretion). EspA, EspB, and EspD are required for translocation of the effector proteins into host cells, in which EspB and EspD are thought to form a pore in the host membrane. Recent study has shown that EspA forms a filamentous structure that assembles as a physical bridge between bacteria and host cell surfaces, which then functions as a conduit for the translocation of bacterial effectors into host cells. To investigate the supermolecular structure of the type III secretion in EPEC, we partially purified it from the bacteria membrane and observed it via transmission electron microscopy. The EPEC type III secretion was composed of a basal body and a needle part and was similar to those of Salmonella and Shigella, except for a sheath-like structure at the tip of the needle. The length of sheath-like structures varied; it extended more than 600 nm and was 10 times longer than the Shigella needle part. The putative major needle component, EspF, was required for both secretion of Esp proteins and needle complex formation. Interestingly, elongation of the sheath-like structure was observed under constitutive expression of EspA but not of EscF. Furthermore, the transmission electron microscopy view with immunogold labeled anti-EspA antibodies clearly showed that EspA is a component of the sheath-like structure. This study revealed, to our knowledge for the first time, the supermolecular structure of the EPEC type III secretion and its direct association with the EspA-sheath-like structure.

Enteropathogenic Enterobacteriaceae coli (EPEC) is a major cause of diarrhea in young children (1). Related pathogens, which cause disease by using similar mechanisms (2), include enterohemorrhagic E. coli O157:H7, rabbit enteropathogenic E. coli, and Citrobacter rodentium, which is found in mice. These pathogens induce a characteristic histopathological lesion, termed an attaching/effacing (A/E) lesion, which is defined by the intimate attachment of bacteria to the epithelial surface and the effacement of host cell microvilli (3). The A/E lesion is mediated by bacteria-host cell interactions, including triggering of host signal transduction pathways and cytoskeletal arrangements (4), and is required for full virulence in vivo (5).

Factors responsible for the A/E lesion formation are encoded by a 35-kilobase pair (kbp) locus termed the LEE (6), which encodes (i) the type III secretion system (the secreton) (7), (ii) the translocated intimin receptor (Tir) (8) and intimin (9), and (iii) Esp proteins. To elicit A/E lesion formation, Tir must be translocated into the host cell membrane via the type III secreton, and then the bacteria adhesion molecule, intimin, directly associates with the translocated Tir (8). Tir-intimin interaction is required for A/E lesion formation and induces accumulations of actin and other cytoskeletal components beneath the attached bacteria (8).

In addition to Tir, three other additional secreted proteins, EspA, EspB, and EspD, are required for formation of A/E lesions and are secreted via the EPEC/enterohemorrhagic E. coli type III secretion. EspA is a structural protein and the major component of a filamentous surface organelle termed “EspA filament” (10). The EspA filament forms a physical bridge between bacteria and the host cell, and it then functions as a conduit for the translocation of bacterial effectors into the host cell. EspB and EspD are thought to be delivered to the host cell membrane, and both proteins showed homology to Yersinia YopB/D proteins, which are believed to form a pore complex in the host membrane and correlate with the ability to induce contact-dependent hemolysis of red blood cells (RBCs) (11, 12).

Recent study has shown that EPEC induces contact-independent hemolysis to RBCs, and EspA filament and EspD are required for this event (13, 14). On the other hand, the espB mutant still caused weak hemolysis, indicating that EspD may be the major component of a translocation pore into the host cell membrane (14). However, the contradictory finding has been presented that the espB mutant showed the nonhemolytic phenotype (13).

Type III secretions are found in many other Gram-negative bacteria species, and they are the mechanism for translocation of bacterial proteins into host cells. Many components of type III secretions show sequence similarities with those of flagellar basal bodies (15). Kubori et al. identified the supermolecular structure termed the “needle complex” (NC) from the Salmonella type III secretion (16), and a similar structure was also identified from Shigella (17, 18), indicating that the type III secretion appears to have universal form. The size of each part of the Shigella NC has been characterized and measured (17); the needle part is 8 nm wide and 45 nm long, and the basal body consists of upper and lower doublet rings with diameters of 15 and 26 nm, respectively, and resembles those of flagella. The height of the basal body is 32 nm, which presumably allows it to traverse both bacterial membranes and the peptidoglycan. The protein components of the NC have been proposed and characterized in both Salmonella (16, 19, 20) and Shigella (17, 18). These studies have revealed that in Salmonella, the basal body is composed of PrgH/K and InvG, and the needle part is composed of PrgI/J. In Shigella, the basal body and needle part are composed of MxiD/G/J and MxiH/I. MxiG, MxiJ, MxiD, MxiH, and MxiJ

Abbreviations: EPEC, enteropathogenic E. coli; NC, needle complex; TEM, transmission electron microscopy; WT, wild type; Tir, translocated intimin receptor; A/E, attaching/effacing.

*K.S. and M.O. contributed equally to this work.

**Present address: Department of Molecular and Cellular Biology, Research Institute for Microbial Disease, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan.

***To whom reprint requests should be addressed. E-mail: akioabe@kitasato-u.ac.jp.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.
show sequence similarity to PrgI, PrgK, InvG, PrgI, and PrgJ, respectively.

Although extensive knowledge has been accumulated about the EPEC type III secreton and EspA filament, the supramolecular structure of the type III secreton and its relationship to EspA are still little understood. Interestingly, no EspA homologue was found in components of type III secretions in Salmonella, Shigella, and other pathogens, except for enterohemorrhagic E. coli. In the EspA-defect strain, EspB and EspD are still secreted into the culture supernatant (21), but they are not able to translocate both proteins into host cells (10, 14), suggesting that the assembly of the EPEC type III secreton may be somewhat different from those of Salmonella or Shigella, and that EspA may be associated with the type III secreton. We report here the identification and characterization of the EPEC type III secreton and its relationship to EspA.

Materials and Methods

Bacterial Strains and Growth Media. EPEC was grown in LB broth or DMEM at 37°C. Shigella was grown in LB broth at 37°C. For details of strains and their phenotypes, see Table 1, which is published as supporting information on the PNAS web site, www.pnas.org.

Cloning and Construction of the Nonpolar Mutant. EPEC escF and espA were cloned into pTrc99A (Amersham Pharmacia) to obtain p99-espA and p99-escF, which constitutively produce EspA and EscF and which were used for complementation of escA and escF mutants. Details of clones and construction of EPEC ΔescF (strain KILS001) can be found in the supporting Methods, which are published on the PNAS web site.

Purification of the NCs from EPEC and Shigella. EPEC and its mutant strains were grown in LB broth overnight at 37°C without shaking, and then overnight cultures were diluted 1:25 in DMEM and incubated for 5 h in a CO2 incubator. Shigella or EPEC was grown in LB broth overnight at 37°C with shaking, and then overnight culture was diluted 1:100 in LB broth and incubated for 2.5 h at 37°C with shaking. Bacteria were then harvested, and the NC was purified by protocols for the partial purification of the Shigella NC (17).

Preparation of Secreted Proteins, SDS-PAGE, and Immunoblotting. Bacteria grown in DMEM or LB were removed by centrifugation (18,000 × g, 10 min), and proteins in the supernatant were precipitated by the addition of ice-cold trichloroacetic acid at a final concentration of 10% and then incubated on ice for 1 h. After centrifugation, the pellets were resuspended in Laemmli sample buffer and analyzed by SDS-PAGE. For immunoblotting, the proteins were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes. EspA, EspB, and EscC were detected with guinea pig polyclonal antibodies against their respective recombinant proteins. Antibodies were purified by affinity chromatography by using respective antigen-immobilized columns.

Infection of Cultured Cells and Immunofluorescence Microscopy. HeLa cells (105) were seeded and grown overnight on 12-mm round glass coverslips, then infected with bacteria for 3 h. Cells were washed three times with PBS and fixed with 3.0% paraformaldehyde in PBS (pH 7.2), then washed three times with PBS. The fixed cells were permeabilized with 20 μl 0.1% Triton X-100 in PBS in the presence of phallloidin–Texas red (to stain filamentous actin) or antiphosphotyrosine antibodies (4G10, Upstate Biotechnology). Alexa-conjugated anti-mouse IgG and IgM (Molecular Probes) were used as the secondary antibody for antiphosphotyrosine. Stained samples were visualized and photographed as described elsewhere (22).

Hemolysis Assay. Bacteria were grown in LB broth overnight at 37°C without shaking, and then overnight cultures were diluted 1:25 into DMEM and incubated for 4 h in a CO2 incubator. They were collected by centrifugation and resuspended in fresh DMEM corresponding to 1/10 volume of the culture media. Rabbit red blood cells (RBCs) were sedimented, washed three times in PBS, and resuspended with DMEM at 107/ml. Equal volumes of bacteria and RBC suspension were mixed together, and each 100-μl aliquot was poured into round-bottom 96-well plates and incubated at 37°C for 90 min in the CO2 incubator. The bacteria–RBC suspensions were gently resuspended with an additional 150 μl of PBS, and then plates were centrifuged. Supernatants (100 μl) were transferred to a fresh plate, where optical density at 550 nm was measured.

Electron Microscopy. Samples were negatively stained with 2% phosphotungstic acid, pH 7.3, that contained 0.2% (wt/vol) sucrose on Butval-98 grids and observed under a JEM 1010 transmission electron microscope (JEOL). For immunolabeling of the NC, samples were applied to Butval-98 grids, fixed with 1% formaldehyde in physiological salt solution, immunolabeled with the affinity-purified anti-EspA polyclonal antibody and with 6-nm colloidal gold-conjugated antibodies against guinea pig IgG (Aurion, Wageningen, The Netherlands) at room temperature for 20 min. After further thorough washing, the NC were fixed and stained as described above.

Results and Discussion

Supermolecular Structure of the EPEC Type III Secreton. To identify the supramolecular structure of the EPEC type III secreton, EPEC was grown in LB broth, and the NC fraction was prepared, negatively stained, and then observed by transmission electron microscopy (TEM). Although we could detect a supramolecular structure similar to that of the Shigella NC, the major components were flagella complexes (Fig. 1A). It has been reported that expression of Esp proteins is affected by environmental conditions such as culture media (23), host body temperature (24), and Congo red (25). Indeed, expression of Esp proteins was induced when EPEC was grown in DMEM but not in LB broth (Fig. 3C). Therefore, the NC fraction was prepared from EPEC wild type (WT) grown in DMEM and observed by TEM (Fig. 1B). On induction in DMEM, the number of flagella complexes was greatly reduced, and the number of NCs with cylindrical symmetry was predominantly increased (white arrow in Fig. 1B). Although the basal body of the NC was reminiscent of that of Shigella (arrows in Fig. 1D), the needle was extraordinarily long and thick (Fig. 1B). To exclude the possibility that the long needle may be specific to the E2348/69 strain (serotype O127), NCs were also prepared from B171–8 (serotype O111) and rabbit enteropathogenic E. coli (REPEC) (serotype O103) strains (Fig. 1C and summarized in Table 1). The TEM view clearly showed that NCs from B171–8 and REPEC had the same shape as that of E2348/69. Our preparation techniques were also confirmed by using Shigella flexneri M94 strain (17), and we found the typical Shigella NCs (arrows in Fig. 1D) that had already been characterized elsewhere (17, 18). To further characterize the needle parts, TEM images of 13 particles were already been characterized elsewhere (17, 18). To further characterize the needle parts, TEM images of 13 particles were
bacterial cell surface when the environmental condition was shifted to an in vivo conditional medium such as DMEM from an in vitro rich medium such as LB media (Fig. 1A and B). These observations may indicate that EPEC has two different phases: (i) a planktonic phase where the flagella complex is used to aid in bacterial swimming; and (ii) a virulent phase where the production of flagella complexes is repressed, and instead the type III secretory protein EscF Is Required for A/E Lesion Formation, Hemolytic Activity, and Secretion.

To investigate whether the NC was an EPEC type III secretory protein, we decided to disrupt a gene encoding EscF. EscF showed homology to the major needle parts of type III secretions in S. typhimurium SPI1 PrgI (24% identity) (19) and Shigella flexneri MxiH (25% identity) (17). In addition, EscF shared homology with other putative type III needle parts of S. typhimurium SPI2 SsaH (35% identity), Pseudomonas aeruginosa PscF (25% identity) (Fig. 2A). The escF located on the LEE4 transcriptional unit in EPEC LEE (26, 27) (Fig. 2C) was amplified by PCR, and the escF mutant strain was constructed as described in the supporting Methods. To analyze whether EscF is involved in A/E lesion formation, HeLa epithelial cells were infected with WT and ΔescF, and then cytoskeletal actin and phosphorylated proteins were labeled with phalloidin–Texas red and fluorescently labeled antiphosphotyrosine antibody, respectively (Fig. 3A). Infection with WT induced the accumulation of actin and tyrosine-phosphorylated proteins beneath the adherent bacteria. In contrast, infection with the escF mutant did not elicit A/E lesion formation. Complementation of ΔescF with the cloned escF in trans restored the ability to form A/E lesions. These results indicate that the lack of cytoskeletal rearrangements could be attributed to the disruption of escF.

A recent study showed that EPEC induces contact-independent hemolysis in RBCs, and that this hemolytic activity depends on the type III secretory protein EspA (14). To confirm the contribution of EscF to the EPEC-induced hemolysis, we measured the hemolytic activity of the escF mutant and compared its activity with WT. The results showed that the escF mutant had reduced hemolytic activity compared to WT, indicating that EscF plays a role in EPEC-induced hemolysis.

Fig. 1. Electron micrographs of negatively stained NC fractions from EPEC and Shigella. The NCs were partially purified from EPEC E2348/69 strain grown in LB broth (A), in DMEM (B), and from EPEC B171–8 grown in DMEM (C, D). (E) Electron micrograph of purified Shigella NC. (F) Alignment of EPEC NCs and comparisons to Shigella. N and B indicate the needle and basal body of EPEC NCs, respectively. Black arrowheads indicate putative immature NCs. Black arrows indicate flagellar complexes (A) and NCs (B, C, and D). Black arrows indicate pilus-like structures. (Bars = 100 nm.)

Fig. 2. Sequence analyses of EscF and EspA and a genetic map of the LEE4. (A) The sequence analyses of needle parts were performed with BLAST (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/). (B) The N terminus of EscF showed homology (13% identity) to the C terminus of EspA (139–190 aa) that contains the coiled-coil region (138–181 aa). The EspA coiled-coil region predicted elsewhere (30) is illustrated (E, β sheet; H, α helix). Note that the leucine-rich heptad repeat of EscF at position Leu1–Leu18 was overlapped with the heptad repeat in EspA coiled-coil region that is required for the A/E lesion formation and EspA-EspA interaction (30). Bold letters indicate identical amino acids. (C) Organization of esp genes and escF in the LEE4 transcriptional unit. An arrow indicates the direction of transcription, and gray boxes indicate genes encoding secreted proteins.
to that of WT (Fig. 3B). Hemolytic activity was observed in WT without close contact to RBCs by centrifugation. In contrast, this activity was completely restored when cloned \( \text{esc}F \) was reintroduced into the \( \text{esc}F \) mutant in trans, indicating that \( \text{EscF} \) is involved in the hemolysis. \( \text{Yersinia} \) (11, 12) and \( \text{Shigella} \) (28, 29) induce hemolytic activities, but centrifugation is required for to evoke hemolysis in RBCs. These findings indicate that the contact-independent hemolysis in EPEC infection can probably be attributed to the extraordinary length of the needle indicating the formation of an EspA filament (10). Indeed, EPEC needle parts varied and were longer than those of \( \text{Shigella} \) (Fig. 1E), thus permitting the elongated needle to be in direct contact with RBCs without centrifugation.

To further investigate the defect caused by \( \text{esc}F \) mutation, EPEC-secreted proteins were analyzed by 12% SDS/PAGE. As shown in Fig. 3C, secretion of EspA, EspB, and EspD was completely blocked by the \( \text{esc}F \) mutation. Complementation of the \( \text{esc}F \) mutant with cloned \( \text{esc}F \) in trans restored secretion of all type III secreted proteins. These results indicate that \( \text{EscF} \) is required for the secretion of Esp proteins, and this observation agrees with secretion-defect phenotypes of mutant strains of \( \text{Shigella mxiH} \) (17, 18) and \( \text{Salmonella prgI} \) (19, 20) that encode major needle components.

Characterization and Assembly of the EPEC NC. We prepared and measured 34 conserved NCs from EPEC WT, and each part was estimated, as shown in Fig. 4A. Although we could not figure out exact sizes of lower and upper rings of the basal body because of the instability in the structure, both rings appeared to be doublet. The widths of the upper and lower rings were estimated to be 16.7 \( \pm \) 1.9 and 18.1 \( \pm \) 2.5 nm, respectively, and the height of the basal body was 31.4 \( \pm \) 4.3 nm. These observations indicate that diameters of the upper and lower rings in EPEC type III secretons are somewhat smaller than those of \( \text{Shigella} \) (Fig. 1E), whereas the height of the EPEC basal body is nearly identical to that of \( \text{Shigella} \). In contrast to \( \text{Shigella} \) and \( \text{Salmonella} \), the EPEC needle is associated with unknown components that seemed to form a sheath-like structure (12.3 \( \pm \) 1.2 nm in width, Fig. 1E). The measured value was wider than the width of the \( \text{Shigella} \) needle [8.0–8.5 nm; Tamano et al. (17) and this study]. Furthermore, minor particles, less than 1% of the total particles, of EPEC NCs appear to have thinner needles (arrowheads in Fig. 1E). Indeed, the NCs possessing the thin needles (termed the neck part in this study), which were located between the sheath-like structure and the basal body, are similar in widths to those of \( \text{Shigella} \). These findings suggest that the sheath-like structure is associated with the surface of the needle part and that the needle structure is extended from the basal body of the EPEC type III secreton.
Next, we prepared NCs from various mutants and observed them by TEM (summarized in Table 1). As expected, the espB mutant did not affect the NC formation. In contrast, NCs were not detected in the type III secretion-defective strain CVD452 (espC mutant strain). As in the case of CVD452, NCs were not detected in the espF mutant, but NCs were restored when cloned espF was reintroduced into the espF mutant in trans. Although the Shigella needle was elongated up to 1 μm when MxiH was overexpressed, we did not observe needle elongation in the espF mutant containing cloned espF, which allows constitutive expression (data not shown). Interestingly, the NC formation was completely abolished by the espA mutant; even secretion of EspB and EspD was not affected by this mutation (Fig. 3C). In contrast, the NC was completely restored when cloned espA was introduced into the espA mutant in trans. To our surprise, the sheath-like structure in this strain appears to be longer than that of the EPEC WT (Fig. 4B). To confirm this observation, we examined the distribution of needle length in 187 randomly chosen NCs from WT (Fig. 4C) and ΔespA containing cloned espA (Fig. 4D). Needle lengths of WT varied, ranging from 32 to 688 nm (average, 93 nm) with a peak at 40–140 nm. Although the peak of the needle length in ΔespA containing cloned espA was similar to that of WT, the needle length was distributed over a wider range, from 32 to 1,360 nm (average, 192 nm), with the maximal size nearly corresponding to the whole bacterial size.

Component of the NC with the Sheath-Like Structure. To further analyze the requirement of EspA for NC formation, we analyzed NC fractions by silver staining 12% SDS/PAGE (Fig. 5A). The NC fraction prepared from WT, ΔespC, and ΔespA appears to contain other components, because unknown pilus-like structures were also observed in this NC fraction (Fig. 1B, NC), and we could not detect drastic changes of NC fractions between WT and mutants. Next, EspA was detected by immunoblotting using anti-EspA antibodies. Although we detect EspA in the bacterial culture supernatant (Fig. 3C), we also detected it in the NC fraction prepared from WT (Fig. 5B). To eliminate the possibility of contamination of bacterial culture supernatants into the NC fraction, immunoblotting was carried out by using antibodies against EspB secreted into culture supernatants. As expected, EspB was not detected in the NC fraction. These results indicate that EspA is specifically localized in the NC fraction. Furthermore, EspA was not detected in the NC fraction prepared from the espF mutant, and the localization of EspA in the NC fraction was partially restored by reintroduction of cloned espF into the espF mutant in trans, indicating that the EspA may be associated with EscF.

EspA Directly Associates with the NC and Is an Essential Component of the Sheath-Like Structure. The study cited above suggests that EspA and EspB interact directly with the type III secretors. To confirm this fascinating hypothesis, NCs purified from EPEC WT were stained with immunogold-labeled anti-EspA antibodies and observed by TEM (Fig. 6). The TEM view clearly showed that the sheath-like structure was attached to the tip of the needle part (an arrow in the left column), and the needle beyond the upper ring is observed from the incomplete NC lacking the lower base (an arrow in the right column). To our surprise, only the sheath-like structure was stained with the anti-EspA antibodies. In contrast, the needle, which was observed as the neck part (Fig. 1E and an arrow in the left column in Fig. 6), and the needle beneath the upper ring (an arrow in the right column in Fig. 6) were not labeled with anti-EspA antibodies. Our TEM view was strongly supported by the finding of Shaw et al. (14), who showed that about 50 nm of the EspA filament close to the bacterial surface was not stained with anti-EspA antibodies. Although we observed an unknown pilus-like structure (black arrow in Fig. 6; Fig. 1B and C), this did not react to the anti-EspA antibodies. These results clearly indicate that EspA is the major component of the sheath-like structure, which is supported by elongation of the sheath-like structure under constitutive expression of EspA (Fig. 4B).
In conclusion, we have shown, to our knowledge for the first time, a direct association of the EPEC type III secreton with EspA. A hypothetical model of the EPEC type III secreton and the sheath-like structure is illustrated in Fig. 7. First, the inner and outer ring components are assembled; a sec-dependent pathway probably requires this step, because the NC was not detected in the espA mutant. Finally, EspA initiates the polymerization from the tip of the needle and assembles the sheath-like structures. The sheath-like structure is expandable, and its elongation is controlled by the amount of EspA (Fig. 4B). The sheath-like structure builds a physical bridge between the bacterium and the host cell membrane, and Tir and other effectors can translocate into host cells.

We thank Brett Finlay (Biotechnology Laboratory, University of British Columbia, Vancouver, BC, Canada), Jim Kaper (Center for Development and Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD), and Michael Donnenberg (Division of Infectious Diseases, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD) for providing the E2348/69 derivatives. We also thank Asaomi Kuwae for critical reading for the manuscript and helpful discussions. This work was supported by operating grants from the Research for the Future Program of the Japanese Society for the Promotion of Science and the All Kitasato Project Study.

1. Donnenberg, M. S. & Kaper, J. B. (1992) Infect. Immun. 60, 3953–3961.
2. DeVinney, R., Gauthier, A., Abe, A. & Finlay, B. B. (1999) Cell. Mol. Life Sci. 55, 961–976.
3. Moon, H. W., Whipp, S. C., Argenzio, R. A., Levine, M. M. & Giannella, R. A. (1983) Infect. Immun. 41, 1340–1351.
4. Rosenshine, I., Donnenberg, M. S., Kaper, J. B. & Finlay, B. B. (1992) EMBO J. 11, 3551–3560.
5. Abe, A., Inukido, U., Hegele, R. G. & Finlay, B. B. (1998) J. Exp. Med. 188, 1907–1916.
6. McDaniel, T. K., Jarvis, K. G., Donnenberg, M. S. & Kaper, J. B. (1995) Proc. Natl. Acad. Sci. USA 92, 1664–1668.
7. Jarvis, K. G., Giron, J. A., Jerse, A. E., McDaniel, T. K., Donnenberg, M. S. & Kaper, J. B. (1995) Proc. Natl. Acad. Sci. USA 92, 7996–8000.
8. Kenny, B., Devinney, R., Stein, M., Reinscheid, D. J., Frey, E. A. & Finlay, B. B. (1997) Cell 91, 511–520.
9. Jerse, A. E., Yu, J., Tall, B. D. & Kaper, J. B. (1990) Proc. Natl. Acad. Sci. USA 87, 7969–7973.
10. Knutton, S., Rosenshine, I., Pallen, M. J., Nisan, I., Neves, B. C., Bain, C., Wolff, C., Dougan, G. & Frankel, G. (1998) EMBO J. 17, 2165–2176.
11. Hakansson, S., Schesser, K., Persson, C., Galoyov, E. E., Rosqvist, R., Homble, F. & Wolf-Watz, H. (1996) EMBO J. 15, 5812–5823.
12. Neyt, C. & Cornelis, G. R. (1999) Mol. Microbiol. 33, 971–981.
13. Warawa, J., Finlay, B. B. & Kenny, B. (1999) Infect. Immun. 67, 5538–5540.
14. Shaw, R. K., Daniell, S., Ebel, F., Frankel, G. & Knutton, K. (2001) Cell Microbiol. 3, 213–222.
15. Huez, C. J. (1998) Microbiol. Mol. Biol. Rev. 62, 379–433.
16. Kubori, T., Matsushima, Y., Nakamura, D., Uraili, J., Lara-Tejero, M., Sukhan, A., Galan, J. E. & Aizawa, S. I. (1998) Science 280, 602–605.
17. Tamano, K., Aizawa, S., Katayama, E., Nonaka, T., Imajo-Ohmi, S., Kuwae, A., Nagai, S. & Sasakawa, C. (2000) EMBO J. 19, 3876–3887.
18. Blocker, A., Jouihri, N., Larquet, E., Goonon, P., Ebel, F., Parrot, C., Sansonetti, P. & Allaoui, A. (2001) Mol. Microbiol. 39, 652–663.
19. Kubori, T., Sukhan, A., Aizawa, S. I. & Galan, J. E. (2000) Proc. Natl. Acad. Sci. USA 97, 10225–10230. (First Published August 15, 2000; 10.1073/pnas.170128997)
20. Kimbrough, T. G. & Miller, S. I. (2000) Proc. Natl. Acad. Sci. USA 97, 11008–11013. (First Published September 12, 2000; 10.1073/pnas.20020497)
21. Kenny, B., Lai, L. C., Finlay, B. B. & Donnenberg, M. S. (1996) Mol. Microbiol. 20, 313–323.
22. Finlay, B. B., Ruschakowski, S. & Dedhar, S. (1991) J. Cell. Sci. 99, 283–296.
23. Kenny, B., Abe, A., Stein, M. & Finlay, B. B. (1997) Infect. Immun. 65, 2606–2612.
24. Abe, A., Kenny, B., Stein, M. & Finlay, B. B. (1997) Infect. Immun. 65, 3547–3555.
25. Abe, A. & Nagano, H. (2000) Microbiol. Immunol. 44, 857–861.
26. Mellies, J. L., Elliott, S. J., Sperandio, V., Donnenberg, M. S. & Kaper, J. B. (1999) Mol. Microbiol. 33, 296–306.
27. Friedberg, D., Umanzki, T., Fang, Y. & Rosenshine, I. (1999) Mol. Microbiol. 34, 941–952.
28. Sansonetti, P. J., Ryter, A., Clerc, P., Maurelli, A. T. & Mounier, J. (1986) Cell 44, 387–396.
29. Blocker, A., Goonon, P., Larquet, E., Niebuhr, K., Cabiaux, V., Parrot, C. & Sansonetti, P. (1999) J. Cell. Biol. 147, 683–693.
30. Delahay, R. M., Knutton, S., Shaw, R. K., Hartland, E. L., Pallen, M. J. & Frankel, G. (1999) J. Biol. Chem. 274, 35969–35974.