Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 4953–4957, July 1989
Cell Biology

Export of an N-terminal fragment of *Escherichia coli* flagellin by a flagellum-specific pathway

GORO KUWAJIMA*†, IKURO KAWAGISHI‡, MICHIO HOMMA‡§, JUN-ICHIRO ASAKA*†, EIJI KONDO*†, AND ROBERT M. MACNAB‡¶

*Shionogi Research Laboratories, Shionogi and Co., Ltd., Fukushima-ku, Osaka 553, Japan; and ‡Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

Communicated by Melvin I. Simon, February 2, 1989

ABSTRACT **Flagellin and several other external components of the bacterial flagellum are thought to be exported, not by the general N-terminal signal peptide-dependent pathway, but by a flagellum-specific pathway involving a central channel in the flagellum itself. We have constructed a variety of mutant alleles of the *Escherichia coli* flagellin gene. Mutant flagellins with large internal deletions or truncations of their C-terminal region could still be exported, even though they could not assemble into filament. The most extreme example was a fragment containing only the N-terminal 183 residues of the 497-residue wild-type flagellin. This result suggests that the N-terminal region of flagellin contains a signal that enables the protein to be recognized and exported by the flagellum-specific pathway.**

The bacterial flagellum of *Escherichia coli* or *Salmonella typhimurium* consists of at least three distinct parts: (*i*) the helical filament that extends some 10 μm from the cell body; (*ii*) the basal body, a structure that consists of four rings and an axial rod and is likely to be part of the rotary flagellar motor; and (*iii*) the hook that connects the filament to the basal body (Fig. 1) (5).

Unlike the eukaryotic flagellum, the bacterial flagellum is not a membrane-enclosed extension of the cytoplasm. Rather, its external components lie beyond the cell membrane. How do these various components reach their final destinations within the organelle? The subunit proteins of the basal body L and P rings (6), which are located in the outer membrane and the periplasmic space, respectively (7), appear to be transported across the cell membrane by the general bacterial export pathway (Fig. 1) (2–4, 34), in which an N-terminal peptide of ≈20 amino acids is cleaved during the export process. In contrast, several of the other components of the flagellum are thought to be exported across the cell membrane by a unique flagellum-specific mechanism (Fig. 1), which has not been well characterized but does not involve cleavage of an N-terminal signal (8, 9). These components include the filament protein (flagellin), the hook protein, the hook-associated proteins [minor components required for assembly of flagellin subunits onto the hook (10)], and probably the component proteins of the basal body rod.

During flagellar morphogenesis, elongation of the filament proceeds in a remarkable fashion by addition of flagellin subunits at its distal end (8, 11). These subunits must therefore travel to an assembly point that can be as far as 10 μm from the surface of the 1-μm cell. It is suspected, though not proved, that the rod and hook also proceed by distal subunit addition. The most likely pathway for this transport is through the central channel of a tubular structure that, as morphogenesis proceeds, progressively consists of the rod, the rod–hook, and the rod–hook–filament. In three-dimensional image reconstructions of flagellar filament (12, 13), such a central channel can be recognized so that the filament is indeed a tubular structure, not a solid one.

As part of an attempt to understand this pathway, we wish to know what aspects of flagellin are important for its successful export.

The middle region of *E. coli* flagellin appears to be dispensable since quite extensive internal deletions can be made and the protein is still exported and, indeed, assembled into filament (14). Some flagellin mutant strains of *S. typhimurium* synthesize a full-size protein, but it remains in the cytoplasm; such mutants have been termed transport or export defective (15). The mutations all occur close to the 3′ end of the flagellin gene, leading to the proposal that the C-terminal region of flagellin may be essential for export. However, we report here that some severely truncated mutant flagellins of *E. coli*, lacking the C-terminal region, are exported just as efficiently as wild-type flagellin.

MATERIALS AND METHODS

Bacteria. *E. coli* strains YK4146 (*fliC*) (16) or KS01 (formerly called C600 *hsm hsr hag*::Tn*10*; ref. 17) were used as the recipients for the various plasmids containing the flagellin structural gene *fliC*.‖ Neither strain synthesizes flagellin, as judged by immunoblotting (15) of samples prepared from whole cells. Transformed cells were grown at 30°C in Luria broth containing ampicillin (50 $\mu g \cdot ml^{-1}$).

Plasmids. Plasmids used are shown in Fig. 2. pKS93 (formerly called pBR322/hag93; ref. 19) (the parent of plasmids pFD2, pFD201, and pFD301) contains the wild-type *fliC* gene with the natural promoter–operator and ribosome-binding sequences intact. pFO3 (the parent of pIK301, pIK302, pIK305, and pIK306) is a pKS93 derivative that retains the wild-type *fliC* gene and regulatory regions but also contains the *tac* promoter upstream. Plasmids pFD2 and pFD301 have small and large in-frame deletions, respectively, of the central region of the gene and have been described (14, 17); both encode a flagellin that can be exported and assembled into a filament that is capable of propelling the cell. pFD201 was obtained by digesting pFD2 first with *Hin*dIII and then with BAL-31, filling with Klenow fragment, and ligating with an 18-mer linker to give the sequence shown in Fig. 3, with a termination codon in its

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.

†Present address: Shionogi Institute for Medical Science, Shionogi and Co., Ltd., Mishima, Settsu-shi, Osaka 566, Japan.
§Present address: Laboratory of Medical Mycology, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan.
¶To whom reprint requests should be addressed.

‖A unified and simplified nomenclature for the flagellar genes of *E. coli* and *S. typhimurium* has been adopted recently (18). *fliC* replaces the symbol *hag* (or *H1* in *S. typhimurium*) for the flagellin structural gene.

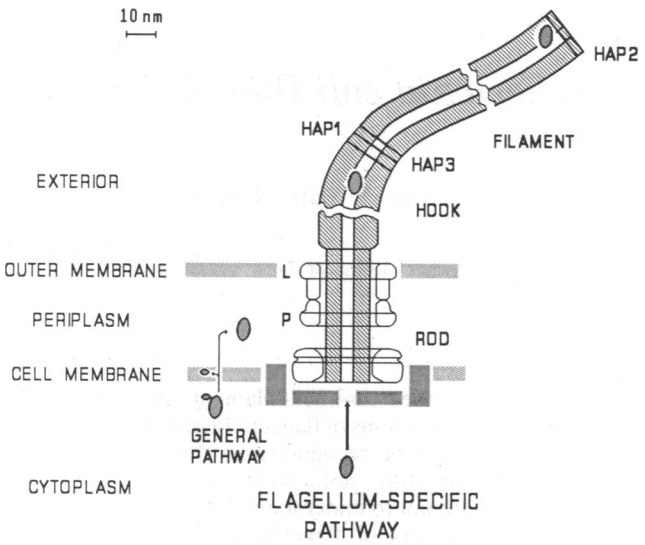

FIG. 1. Export pathways for the components of the bacterial flagellum that are external to the cell membrane. The known external components are the basal body rod, the outer rings of the basal body, the hook, the hook-associated proteins (HAPs), and the filament. The inner rings of the basal body are not external to the cell, nor are the switch and Mot proteins (1), which have not been identified morphologically and are shown as shaded rectangles. The component proteins of the outer (P and L) rings, which lie in the periplasmic space and the outer membrane, have a consensus signal sequence that is cleaved during the export process (2, 3, 34); they are therefore believed to use the general bacterial export pathway (4). The axial components (hatched area) do not have a cleaved signal sequence, and—at least in the case of the filament protein flagellin—are added at the distal end of the growing structure. They therefore use a flagellum-specific pathway, which is believed to consist of a central channel through the rod, hook, hook-associated proteins, and filament. The subunits must pass through this channel to reach their final destination.

linker region; it encodes a 240-residue protein consisting of the first 238 residues of flagellin and two artificial residues, and it may also encode (as a separate fragment) the last 242 residues of flagellin as a result of a fortuitous ribosome binding site (20) and start codon. In pIK302, a 240-base-pair (bp) *Sac* II fragment near the 3′ end of the gene has been deleted (in-frame), and so the gene encodes an internally deleted polypeptide with 417 residues of wild-type flagellin. pIK305 has an out-of-frame *Pst* I deletion in the latter half of the gene and encodes a protein consisting of the N-terminal 293 amino acid residues of flagellin and 15 artificial residues. pIK306 has a 4-bp insertion into a *Spe* I site in the first half of the gene, which generates an in-frame stop codon; the plasmid encodes a protein consisting of the N-terminal 183-amino acid residues of flagellin. pIK301 has the *Sac* II fragment, which was deleted in pIK302, placed in the opposite orientation from the natural sequence; since this inversion does not generate a stop codon, pIK301 encodes a mutant protein that has 79 artificial residues substituting for the natural sequence (Table 1).

Chemicals and Enzymes. These were obtained from standard commercial sources.

Manipulation of DNA. Plasmid constructions, manipulation of DNA, and transformation of host cells were all carried out by standard procedures. DNA sequencing of the linker region in plasmid pFD201 was carried out by the dideoxy nucleotide chain-termination method (21) using M13mp18 phage.

Motility Assay. The ability of a plasmid to confer motility on the transformed host was assayed by inspection for swarming on semisolid plates as described (14). The presence or absence of filaments on cells grown in liquid medium was

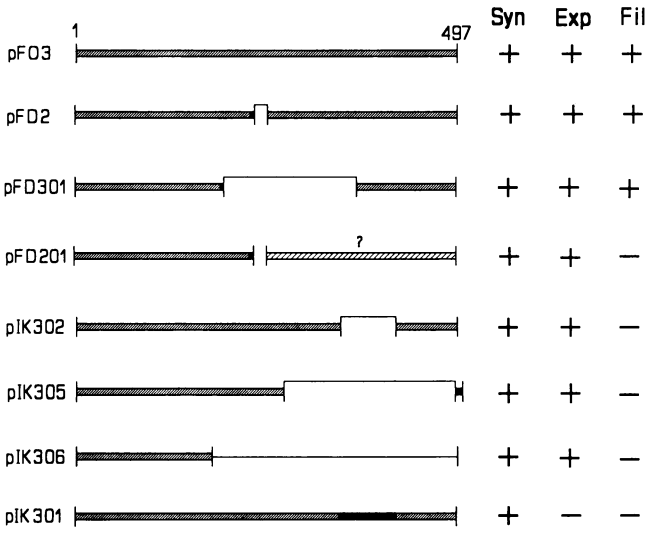

FIG. 2. Plasmids containing wild-type (pKS93 or pFO3) and mutant alleles of the *E. coli* structural gene for the flagellar filament protein, flagellin. The plasmids also contain the natural promoter–operator and ribosome binding site sequences. The nature of each allele is shown, along with its properties with respect to flagellin synthesis (Syn), export (Exp) (cf. Fig. 4), and ability to assemble into filament and thereby confer motility (Fil). Natural coding sequence is indicated by hatched bars; artificial coding sequence is shown by solid bars; fusions across a deletion are indicated by bridging lines; DNA that is not thought to be coding is shown by single lines. Synthesis of the C-terminal fragment of pFD201 flagellin (?) has not been demonstrated but is possible on the basis of the adjacent sequence (Fig. 3). The constructions are described in more detail in the text.

also established by high-intensity dark-field light microscopy (22).

Export Assay. Transformed cells were grown overnight, Vortex mixed at full power (Lab-Line Instruments Super-Mixer) for 2 min to shear any attached filaments, and centrifuged (5000 × *g* for 10 min) to pellet the cells. A 30-μl sample of the supernatant was analyzed directly (without prior concentration by ultrafiltration as was done in ref. 14) by SDS/PAGE (23) and Coomassie staining. The concentration of a given protein in the culture medium was estimated by densitometry of the gel using purified flagellin as a standard.

Immunoblotting. Pelleted cells or culture supernatants were subjected to SDS/PAGE, transferred to nitrocellulose, and immunoblotted with polyclonal antibody raised against monomeric flagellin as described (15).

Protein Purification and N-Terminal Sequence Analysis. The 31-kDa protein exported by KS01 cells transformed with pFD201 was purified as follows: the relevant section of a gel containing the protein was excised, electroeluted, and applied to a reverse-phase HPLC column (Aquapore RP300, Brownlee Lab). The column was then washed with 0.1% trifluoroacetic acid and eluted with a 0–100% gradient of acetonitrile. A single band of 31-kDa protein was detected by

```
                    HindIII  SmaI   BglII
...GGTGGTGCAAGCTAAGCTTCCCGGGAGATCTTGGTGACAATG...
...GlyGlyAlaSer *          S-D      MetThrMet...
      238                           Val
```

FIG. 3. Nucleotide sequence of the linker region of plasmid pFD201, which disrupts the *E. coli* flagellin gene. Artificially introduced DNA and amino acid sequence are shown in italics. As well as introducing a termination codon, the construction offers a potential in-frame restart because of the sequence GGAG (S-D, Shine–Dalgarno sequence) 6 bp upstream from a GTG codon.

Table 1. Deduced amino acid composition of the 79-residue artificial sequence of pIK301 and that of the corresponding wild-type sequence

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
pIK301	9	6	1	1	2	0	4	1	0	2	6	2	0	4	11	16	6	0	1	7
Wild type	9	2	4	11	0	2	2	11	0	2	9	6	0	1	1	5	7	0	1	6

The wild-type amino acid composition is based on the translated sequence of the *Sac* II fragment of the flagellin gene (19); that of pIK301 flagellin is based on translation of the inverse complement of the same fragment.

SDS/PAGE. Amino acid sequence analysis of the N-terminal region of pFD201 flagellin was performed by the gas-phase method (Applied Biosystems, model 470A).

RESULTS

Export of a 31-kDa Flagellin Fragment. *E. coli* flagellin is a 51,172-Da protein with 497 amino acid residues (Fig. 2) (19). Plasmid pFD2 is a plasmid we had constructed previously (17), which contains the flagellin structural gene, *fliC*, mutated as a result of a small deletion and insertion of a *Hin*dIII linker in the central region of the gene; it also contains the flanking transcriptional and translational control regions of the gene. This mutant allele supports flagellation and motility. A derivative of this plasmid, pFD201, has a termination codon introduced into the linker region and should encode a 25,094-Da protein consisting of only the N-terminal 238 residues of wild-type flagellin plus two artificial residues (Figs. 2 and 3). Nonflagellate hosts carrying a mutation in the flagellin structural gene (*fliC*) remained nonflagellate when transformed with pFD201. However, they exported a protein of apparent molecular mass 31 kDa in amounts such that it was the major protein component in the culture medium (Fig. 4*A*, lane b). The concentration of the 31-kDa protein was estimated at 37 $\mu g \cdot ml^{-1}$, comparable to the amount of wild-type flagellin (10 $\mu g \cdot ml^{-1}$) exported by mutants that (because of a defect in one or other of the hook-associated proteins) cannot assemble flagellin into filament (25). We purified this 31-kDa protein and determined the sequence of the N-terminal 24 residues. Since these were identical to those of wild-type flagellin (19), we conclude that it was the pFD201-encoded mutant flagellin.

Thus, the N-terminal 238 residues of flagellin (plus two artificial residues) constitute a protein that can be exported as efficiently as the 497-residue wild-type protein. However, since plasmid pFD201 contains a potential in-frame restart immediately following the reading frame for the N-terminal fragment (Figs. 2 and 3), the possibility remained that virtually the entire flagellin protein was being synthesized—in two fragments—and that the 242-residue C-terminal fragment was in some way facilitating the export of the N-terminal fragment, even though it itself was not being exported. We therefore constructed other mutant alleles in which the possibility of a C-terminal helper fragment did not exist.

Export of Other Mutant Flagellins. Various plasmids with mutant flagellin alleles were constructed and tested for whether the resultant proteins could be synthesized, exported, and assembled into filament to confer motility (Fig. 2). For reference, we examined (*i*) in-frame deletions of the central region of the gene, which were known (14, 17) to encode functional flagellins (pFD2 and pFD301 flagellins). The other mutant alleles included ones with (*ii*) an in-frame deletion within the 3′ third of the gene, resulting in an internal deletion within the C-terminal region of the protein (pIK302 flagellin), (*iii*) a large deletion of most of the 3′ half of the gene (pIK305 flagellin), (*iv*) a frame-shift mutation resulting in

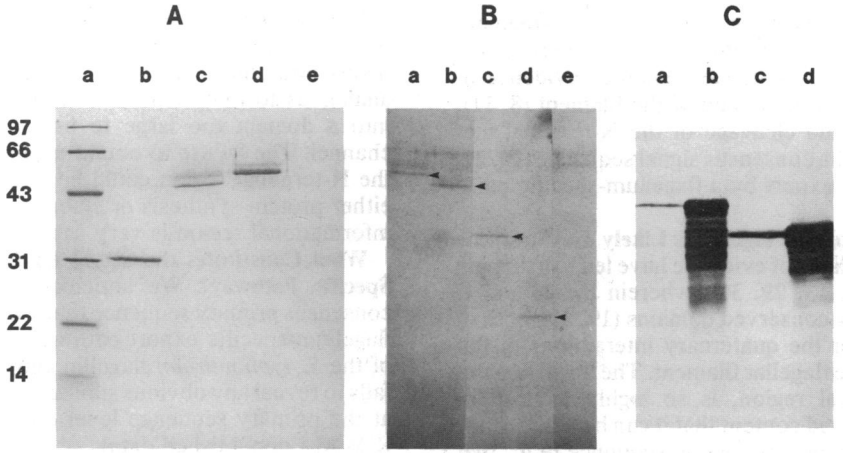

FIG. 4. Export of mutant flagellins by cells transformed with the plasmids described in Fig. 2. (*A*) Export by pFD201-transformed cells. In Coomassie-stained SDS/polyacrylamide gels of the culture medium supernatant, a single major band of severely truncated flagellin with an apparent molecular mass of 31 kDa was detected (lane b). For comparison, the amount of flagellin incorporated into filament by cells transformed with a plasmid encoding a flagellin that has a small internal deletion (pFD2; lane c; cf. ref. 14), or wild-type flagellin (pKS93; lane d) is shown. No major band was detected with pBR322-transformed cells (lane e). The bands above the position of wild-type flagellin might on the basis of their mobility be hook-associated proteins 1 and 2 (cf. ref. 24). Standards (lane a) are phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.7 kDa), carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa). (*B*) Export of mutant flagellins by other transformed cells, containing the following plasmids: lane a, pFO3; lane b, pIK302; lane c, pIK305; lane d, pIK306; lane e, pIK301. A single band of the expected mobility was seen (arrowheads) in all cases except for cells transformed with pIK301, whose flagellin has a large artificial replacement in its C-terminal region (see Figs. 2 and 3). (*C*) Immunoblotting of samples with polyclonal anti-flagellin antibody. The exported proteins in *A* and *B*, as well as proteins of the corresponding size in whole-cell samples, were all tested and found to cross-react (data not shown), supporting their identity as mutant flagellins. Shown here are samples from cells transformed with pIK301 (lanes a and b) and pIK302 (lanes c and d). Whole-cell (lanes b and d) and supernatant (lanes a and c) samples derive from the same culture volume and therefore represent directly the distribution of material between the two fractions. Both synthesize cross-reacting material in comparable amounts (lanes b and d), but the amount exported by pIK301-transformed cells (lane a) is greatly reduced compared to pIK302-transformed cells (lane c). Because of different electrophoretic conditions, band positions in *C* do not correspond to those in *A* and *B*.

termination about one-third of the way into the gene (pIK306 flagellin), and (*v*) an inversion that maintained an open reading frame encoding a protein with a large artificial replacement (Table 1) toward its C-terminal end (pIK301 flagellin).

The plasmids were introduced into a nonflagellate host (Fig. 2). Except for those transformed with pFD2 or pFD301, none of the transformants was motile or even had filaments. However, with the single exception of cells transformed with pIK301, each exported into the culture medium one major protein, whose electrophoretic mobility corresponded to that predicted for the relevant mutant flagellin (Fig. 4*B*). The most extreme case was pIK306, which encodes a 20-kDa N-terminal fragment consisting of only 183 residues. That these proteins were mutant flagellins was verified by immunoblotting with polyclonal anti-flagellin antibody. (Presumably because of extensive loss of the central region in pFD201 and pIK306 flagellin, the antigenicity was substantially reduced compared to full-length flagellin.) By blotting samples of whole-cell protein as well as of the culture medium supernatant, we were able to establish that all strains, including those transformed with pIK301 (Fig. 4*C*), were capable of synthesizing their respective mutant flagellins. Because of its greater sensitivity, the immunoblotting assay also revealed that pIK301 transformed cells did appear to be capable of exporting mutant flagellin, but in amounts at least 10-fold below those of the other strains.

DISCUSSION

N-Terminal Recognition Does Not Imply Use of the General Export Pathway. We have shown that a 183-residue N-terminal fragment (pIK306 flagellin), which is just a little over one-third the size of the wild-type protein, can nonetheless be exported from the cell. Various other mutant flagellins with extensive removal of C-terminal information were also exported, and we do not yet know the lower size limit of an exportable sequence. Export of an N-terminal fragment might lead one to suspect that flagellin uses the general signal peptide-dependent pathway, but the combined evidence of addition of subunits at the distal end of the filament (8, 11), lack of processing beyond cleavage of the N-terminal methionine (26), and lack of a consensus signal sequence (19, 27, 28), argues strongly for export by a flagellum-specific pathway (Fig. 1).

The N-Terminal Region of Flagellin Is Likely To Constitute a Domain. A number of lines of evidence have led to a domain model for flagellin (12, 13, 29, 30), wherein the N and C termini constitute highly conserved domains (19, 27), both of which are necessary for the quaternary interactions of flagellin subunits within the flagellar filament. The third domain, consisting of the central region, is so highly tolerant of variation both in length and content that it can be deleted (14, 17) or replaced by a completely foreign sequence (e.g., that of lysozyme or cholera toxin; refs. 31, 35) and still provide normal function in terms of assembly into helical filament and propulsion of the cell. This central region is the main antigenic region of flagellin (28, 29, 32) lying at the surface of the flagellar filament.

The fact that the N-terminal region alone can be recognized by the transport apparatus suggests it may possess some well-defined structure and provides further support for the domain model of flagellin.

It seems clear from the extreme tolerance within the central domain that it does not contribute either to important structural features or to information for export. We do not yet know whether the C-terminal sequence of flagellin contains information necessary for its export or whether it is dependent on the N-terminal sequence in this regard.

An Artificial C-Terminal Sequence Inhibits Export. The importance of conformation in export via the signal peptide-dependent pathway has been demonstrated (for example, see ref. 33). Our results suggest that this may be true of the flagellum-specific export pathway also. The finding that large deletions of C-terminal sequence can be tolerated with respect to export (pFD201, pIK302, pIK305, and pIK306 flagellins), but that a substitution (pIK301 flagellin) blocks the process, implies that the wrong sequence is worse than lack of the right sequence. The artificial replacement in pIK301 flagellin differs drastically from wild type in amino acid composition (Table 1) as well as sequence, and there can be little doubt that this will disrupt the normal higher-order structure of the protein. To cite two examples: (*i*) The 79-residue artificial sequence contains 11 proline residues, 6 of them as adjacent pairs, whereas the corresponding wild-type sequence has only one. (*ii*) Wild-type flagellin from *E. coli* (and other species) lacks cysteine entirely, whereas the artificial sequence has two cysteine residues, which could form a disulfide bridge.

The deleterious effect of the wrong C-terminal sequence might be a result of the C-terminal domain interacting with the N-terminal domain and altering the structure of the latter in a manner that affects export. Alternatively, it might be that a full-length but wrongly folded C-terminal domain has too large a diameter to travel down the channel (which appears quite narrow by electron microscopy) and therefore prevents the N-terminal domain from doing so. It will be interesting in this regard to examine whether the failure of pIK301 flagellin to be exported is dominant—i.e., whether it can competitively inhibit export of wild-type flagellin.

C-Terminal Flagellin Defects in Spontaneous Export-Defective Mutants. Among spontaneous mutants that were defective in export of flagellin, the defects invariably appeared in the C-terminal region of the protein (15). This contrasts with our findings (*i*) that the N-terminal region contains sufficient information for its own export and (*ii*) that at least some massive alterations of the C-terminal region do not affect the export process. We suspect that the spontaneous mutations in the C-terminal region may be functionally analogous to that of pIK301 flagellin, causing poor folding into a domain too large to be accommodated within the channel. The failure to obtain export-defective mutations in the N-terminal region could be because they interfere with either protein synthesis or stability or because the essential informational region is very small.

What Constitutes the Signal for Export by the Flagellum-Specific Pathway? We anticipated that there might be a consensus primary sequence that would act as a signal for the flagellum-specific export pathway. However, a comparison of the *S. typhimurium* flagellin and hook protein sequences fails to reveal any obvious similarity that might reflect a signal at the primary sequence level (D. J. DeRosier, M.H., and R.M.M., unpublished data).

Thus, while we have shown that the 5′ coding region of the flagellin gene, together with its promoter–operator region and ribosome binding site, contains the information necessary for the export of an N-terminal flagellin fragment, we cannot at this point say which parts of the sequence are essential.

This work has been supported by U.S. Public Health Service Grant AI12202 (to R.M.M.).

1. Macnab, R. M. & DeRosier, D. J. (1988) *Can. J. Microbiol.* **34**, 442–451.
2. Homma, M., Komeda, Y., Iino, T. & Macnab, R. M. (1987) *J. Bacteriol.* **169**, 1493–1498.
3. Homma, M., Ohnishi, K., Iino, T. & Macnab, R. M. (1987) *J. Bacteriol.* **169**, 3617–3624.

4. Oliver, D. (1985) *Annu. Rev. Microbiol.* **39**, 615–648.
5. DePamphilis, M. L. & Adler, J. (1971) *J. Bacteriol.* **105**, 384–395.
6. Jones, C. J., Homma, M. & Macnab, R. M. (1987) *J. Bacteriol.* **169**, 1489–1492.
7. DePamphilis, M. L. & Adler, J. (1971) *J. Bacteriol.* **105**, 396–407.
8. Iino, T. (1969) *J. Gen. Microbiol.* **56**, 227–239.
9. Iino, T. (1974) *J. Supramol. Struct.* **2**, 372–384.
10. Homma, M., Kutsukake, K., Iino, T. & Yamaguchi, S. (1984) *J. Bacteriol.* **157**, 100–108.
11. Emerson, S. U., Tokuyasu, K. & Simon, M. I. (1970) *Science* **169**, 190–192.
12. Shirakihara, Y. & Wakabayashi, T. (1979) *J. Mol. Biol.* **131**, 485–507.
13. Trachtenberg, S. & DeRosier, D. J. (1987) *J. Mol. Biol.* **195**, 581–601.
14. Kuwajima, G. (1988) *J. Bacteriol.* **170**, 3305–3309.
15. Homma, M., Fujita, H., Yamaguchi, S. & Iino, T. (1987) *J. Bacteriol.* **169**, 291–296.
16. Komeda, Y., Kutsukake, K. & Iino, T. (1980) *Genetics* **94**, 277–290.
17. Kuwajima, G. (1988) *J. Bacteriol.* **170**, 485–488.
18. Iino, T., Komeda, Y., Kutsukake, K., Macnab, R. M., Matsumura, P., Parkinson, J. S., Simon, M. I. & Yamaguchi, S. (1988) *Microbiol. Rev.* **52**, 533–535.
19. Kuwajima, G., Asaka, J.-I., Fujiwara, T., Fujiwara, T., Node, K. & Kondo, E. (1986) *J. Bacteriol.* **168**, 1479–1483.
20. Shine, J. & Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1342–1346.
21. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
22. Macnab, R. M. (1976) *J. Clin. Microbiol.* **4**, 258–265.
23. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
24. Homma, M. & Iino, T. (1985) *J. Bacteriol.* **164**, 1370–1372.
25. Homma, M., Fujita, H., Yamaguchi, S. & Iino, T. (1984) *J. Bacteriol.* **159**, 1056–1059.
26. Joys, T. M. & Rankis, V. (1972) *J. Biol. Chem.* **247**, 5180–5193.
27. Joys, T. M. (1985) *J. Biol. Chem.* **260**, 15758–15761.
28. Wei, L.-N. & Joys, T. M. (1985) *J. Mol. Biol.* **186**, 791–803.
29. Yamaguchi, S., Fujita, H., Sugata, K., Taira, T. & Iino, T. (1984) *J. Gen. Microbiol.* **130**, 255–265.
30. Fedorov, O. V. & Kostyukova, A. S. (1984) *FEBS Lett.* **171**, 145–148.
31. Kuwajima, G., Asaka, J.-I., Fujiwara, T., Fujiwara, T., Nakano, K. & Kondo, E. (1988) *Bio/Technol.* **6**, 1080–1083.
32. Horiguchi, T., Yamaguchi, S., Yao, K., Taira, T. & Iino, T. (1975) *J. Gen. Microbiol.* **91**, 139–149.
33. Randall, L. L. & Hardy, S. J. S. (1986) *Cell* **46**, 921–928.
34. Jones, C. J., Homma, M. & Macnab, R. M. (1989) *J. Bacteriol.* **171**, in press.
35. Newton, S. M. C., Jacob, C. O. & Stocker, B. A. D. (1989) *Science* **244**, 70–72.