Bacterial flagellar diversity and significance in pathogenesis

Charles W. Penn and Catherine J. Luke

School of Biological Sciences, University of Birmingham, Birmingham, UK

Received 19 June 1992
Accepted 30 July 1992

Key words: Flagella; Helicobacter; Pathogenicity; Spirochaete; Treponema

1. SUMMARY

Bacterial flagella are structurally diverse, ranging from the thoroughly investigated model examples found in Escherichia coli and Salmonella typhimurium to the more exotic sheathed flagella of, for example, Helicobacter pylori, and the complex multi-flagellin endoflagella found in many spirochaetes. We summarize some of the emerging structural and genetic findings relating to these more novel flagellar types, and outline their possible significance in the pathogenicity of some medically important bacteria.

2. STRUCTURE OF BACTERIAL FLAGELLA

There is wide variation among bacterial species in the number, arrangement, protein composition and fine structure of their flagella, although the majority of studies have been on Salmonella typhimurium and Escherichia coli [1]. The major structural component of flagella is the filament. In S. typhimurium it has been analysed by X-ray diffraction techniques, and the pattern of subunit arrangement determined [2]. This approximates to hexagonal close packing, of ‘flagellin’ protein subunits, stacked in a complex array to form a helix. Subunits are arranged in a series of 11 parallel columns or fibrils, which, despite the identical nature of the subunit proteins, are not identical in their conformation, thus leading to a helical three-dimensional structure rather than a straight cylinder. In the best explored examples there is a single flagellin in the filament, as in E. coli and S. typhimurium, in which it has an Mr of about 51 K [1]. The filament may also contain different protein species, as in the periplasmic flagella of Treponema pallidum [3], T. phagedenis [4], and T. denticola [5] which contain at least two proteins of Mr values in the region of 37–39 K and 31–34 K (see below).

At the base of the filament is the hook, which is believed to function as a universal coupling. This is composed of a protein distinct from the filament flagellin. Several other proteins, known as hook associated proteins, are found both at the
filament tip or cap, and at its junction with the hook [1]. In *S. typhimurium*, spanning the outer membrane, peptidoglycan layer, periplasm and cell membrane, is a rod-like structure, and associated inner and outer rings. Together, these structures make up the ‘basal body’ of the flagellum. There may be additional structures present in association with the basal body; for example, in *Wolinella succinogenes*, a bacterium isolated from the bovine rumen, a basal disk in the periplasm and a ring-like structure associated with an elongation of the basal body extending into the cytoplasm have been identified [6]. A similar structure to the basal disk has been reported in *H. pylori* [7].

It is also interesting to note that, in *W. succinogenes*, there is a depression in the cell wall at the flagellar insertion site; a feature that has also been observed in *H. pylori* (our unpublished observations; see Fig. 1a), which may reflect the close relationship between these organisms [8].

Some species of bacteria possess sheathed flagella, in which the sheath appears ultrastructurally to be a membrane-like, relatively flexible covering around the flagellum. For example, *Vibrio parahaemolyticus* produces a single, polar, sheathed flagellum, but only when it is growing in semi-solid or liquid medium: its ‘swimmer phase’. When grown on a solid medium, *V. parahaemolyticus* produces multiple, lateral flagella which are unsheathed [9]. *H. pylori* typically has between one and six polar flagella [10] covered by a sheath, which may or may not be a continuation of the outer membrane (see, for example, Fig. 1b which suggests a lack of continuity between outer membrane and sheath). Putative flagellar sheath polypeptides distinct from the major flagellin have been identified [11], but whether or not these are also distinct from outer membrane components remains to be determined. In some preparations, it appears that the flagella of *H. pylori* terminate in a bulb structure [12]. This has also been observed in *H. cinaedi* and *H. fennelliae* (formerly *Campylobacter cinaedi* and *Campylobacter fennelliae*, respectively), but it may be an artefact of staining for electron microscopy ([13]; C.J. Luke, unpublished, and Fig. 1b). In *Vibrio cholerae*, there is evidence that outer membrane components are present in the flagellar sheath [14].

In the spirochaetes, the flagella resemble those of other bacteria in terms of basic structure, but they are enclosed between the outer membrane sheath and the protoplasmic cylinder [15], and hence are named ‘periplasmic flagella’ or ‘endo-flagella’. In many spirochaete genera, but not all, flagella comprise an inner core (30–35 K subunits) and an outer layer (37–39 K subunits) sometimes referred to as a sheath but not to be confused with the membranous flagellar sheath described above. It appears that the flagellar outer layer of treponemes is able to modify the curvature of the flagellum (as seen in Fig. 1c, in which flagella of *T. denticola* abruptly assume an almost straight appearance at the point where the outer layer terminates). In *Borreliae* only a single flagellin protein is found, and, for example in *B. burgdorferi*, it has an *M*<sub>r</sub> of 41 K [16]. Protruding endoflagella encased in membrane material, but not confined to the axis of the spiral body of the organism, have also been observed in several spirochaetes, e.g. *T. denticola*, *T. phagedenis* and *B. burgdorferi* [17]. These protrusions have the same helix handedness, pitch and diameter as purified periplasmic flagella, and are thought to provide the bacteria with an alternative mode of motility as an adaptation to different environmental conditions. In some circumstances an appearance resembling that of the true sheathed flagella may be seen in spirochaetes, in which the

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**Fig. 1.** (a) A negatively stained cell of *Helicobacter pylori*, showing a distinct depression which is sometimes present at the pole where flagella emerge. (b) A negatively stained cell of *Helicobacter pylori*, illustrating a ‘terminal bulb’ structure (tb) of a sheathed flagellum, which appears to be a fluid extension of membranous material beyond the distal end of the rigid flagellum which is concealed within the sheath; there appears to be a discontinuity between the outer membrane of the cell and the flagellar sheath (arrow); a fragment of non-sheathed flagellar filament is also present. (c) A bundle of endoflagella within an outer membrane protrusion of *Treponema denticola*. At the point of transition from the thick, outer layered complete flagellar filament to the thinner core (arrows), the waveform of the flagella abruptly changes. (d) Protruding flagella of *T. denticola* surrounded by outer membrane protrusions, with the appearance of sheathed flagella. Bars represent 0.5 μm.
membrane around the flagellum is presumably an extension of the outer membrane (Fig. 1d), and suggests a possible common morphogenetic process with that of other sheathed flagella.

3. GENETIC ANALYSIS OF FLAGELLAR COMPONENTS AND ASSEMBLY

Genetic analysis has been an extremely powerful tool in elucidating the structure and mechanism of assembly of bacterial flagella, notably in the laboratory of R.M. Macnab [18]. The most detailed analysis has been confined to *E. coli* and *S. typhimurium*, but it seems likely that basic features will be shared with the more exotic examples discussed here. The existence of more than 40 flagellar genes and their role in the overall ordered assembly of the complex structure of the flagellum make this one of the most complex gene families in bacteria. A notable feature is the grouping of genes and operons so that their expression is largely coordinated with the order of assembly of the flagellum, in an efficient 'cascade' so that the last components to be added to the structure (at the distal end of the flagellum) are not expressed unless there is a base to which these late components can be added.

The basic features of flagellar gene organisation and function in *E. coli* and *S. typhimurium* (abbreviated below as ‘the enteric bacteria’) are as follows (flagellar gene nomenclature in these species was recently revised [19] but for the other organisms discussed below we will retain the nomenclature used by the authors concerned). There are three main regions of flagellar and associated genes in the enteric bacteria: region I (*flg*) encodes mainly components of the basal unit and hook; region II (*flh*) encodes chemotaxis and motor functions as well as containing regulatory genes including the key *flhC* and *flhD* ‘master’ regulators necessary for expression of the entire cascade; region IIIa (*fli*) includes another key regulatory gene *fliA* which probably encodes an alternative sigma factor required for expression of late components and is of the *σ28* type; and both regions IIIa and IIIb contain numerous other late component structural genes including the flagellin gene itself, *fliC*. In *S. typhimurium* only, a fourth region, *flj*, contains the additional copy of the flagellin gene involved in classical flagellar antigen phase switching.

The genetics of flagellar assembly in pathogenic bacteria other than the enteric bacteria has received little attention in comparison, but some features are known. In *Campylobacter*, flagellin genes have been cloned and sequenced [20–22], and in both *C. jejuni* and *C. coli* tandem duplicate copies have been described. The 5' copy *flaA* (homologue of *fliC* of the enteric bacteria) appears to be the normally expressed structural flagellin; the downstream copy *flaB* appears to be transcribed only in *C. coli* and at a lower level than *flaA*, and its exact function is not known [20]. Interestingly, a more recently published *Campylobacter* flagellin sequence [23] appears significantly different from those first published, suggesting considerable heterogeneity within the genus in flagellar genes. The expression of the *flaA* genes appears to be dependent on a *σ28*-type promoter. In *H. pylori*, recent data suggest that there is, in contrast to the closely related *Campylobacters*, only one flagellin gene [24]; however there is also evidence of both antigenic and molecular mass heterogeneity in native flagella [25] and further data are awaited with interest.

Gene cloning has provided some fascinating information about treponemal flagellar genes, and this work emphasises bacterial flagellar structural as well as genetic diversity. Recently, genes for the multiple polypeptides of treponemal flagella have been cloned and sequenced [26–29] and some unexpected results have emerged. Proteins previously assumed to be homologues of the flagellins of the enteric bacteria appear to differ in their genetic expression. The *flaA* gene of *T. pallidum*, which encodes the major polypeptide of the flagellar filament outer layer, has a promoter sequence of *σ70* type [29], suggesting that its transcriptional control is separate from that of the *flaB* genes, which encode core polypeptides of the flagellum and which have typical *σ28* promoter sequences, like those of the late flagellar structural genes of the enteric bacteria. Furthermore, the sequences of the *flaA* and *flaB* genes of *T. pallidum* [27,29] indicate that while the FlaB
proteins appear to lack export-related signal peptides, as do the structural flagellar proteins of the enteric bacteria which are exported via the central channel in the flagellar structure, the FlaA protein (in contrast) has a typical export signal peptide. The FlaA protein, therefore, is likely to be exported from the cytoplasm into the periplasm and then added to the outer layer of a conventionally constructed flagellum made from one or more FlaB flagellins (we do not know at present whether more than one FlaB protein is present in every flagellar filament or whether there is heterogeneity between individual flagella). In any event these are striking differences from the enteric bacterial model of flagellar assembly. The reasons for these differences might include a need for a specialized flagellar surface in order for it to function within the periplasm.

4. ROLE OF FLAGELLA AND MOTILITY IN PATHOGENICITY

Many pathogenic bacteria possess flagella, and their role in motility of these bacteria is important in colonisation. Aflagellate or non-motile variants of pathogenic species of bacteria are often unable to establish infections in either animal models or human cells cultured in vitro. For example, aflagellate variants of H. pylori, the bacterium associated with gastritis and peptic ulceration in humans, were, in contrast to flagellate organisms, unable to colonise the gastric mucosa in gnotobiotic piglets [30]. It is believed that the motility of T. pallidum, the aetiological agent of venereal syphilis, contributes to its ability to invade host tissue [31]. Non-motile flagellate strains and strains lacking a flagellar filament, as well as aflagellate strains, of C. jejuni, were all cleared from the intestine rapidly following infection in experimental animals [32]. This suggests that it is not only motility that is necessary for successful establishment of infection by C. jejuni, and more recent studies show that the flagella may, in their own right, have a significant role in pathogenesis [33,34]. Pseudomonas aeruginosa requires flagella and motility to invade and disseminate within the host in the burned mouse model, although non-flagellate, non-motile variants could still colonise the wound site [35]. An aflagellate mutant of B. burgdorferi, the Lyme disease spirochaete, showed reduced ability to penetrate human endothelial cell layers in vitro [36].

In species in which the flagella are sheathed, it is not understood how, if at all, the sheath contributes to the pathogenic process. It is conceivable that, if the sheath is continuous with the outer membrane, it may bear molecules involved in the initial interaction between the bacterium and host cells, such as adhesins, although there could possibly be functional conflict in the case of an organ of motility also serving as an adhesion structure. It has been proposed that the flagellar sheath of H. pylori serves to protect the flagellin from gastric acid since, like other bacterial flagella, those of H. pylori are easily depolymerised in acidic conditions [37].

There is substantial evidence, then, that flagella contribute significantly to establishment of infection by pathogenic bacteria. They also feature prominently as immunogens in the host immune response to these infections, and are proving to be significant antigens in newly emerging diagnostic tests for several important bacterial diseases. Basic studies on flagellar structure, function and genetics, and especially of their diversity in some of the newly emergent and the more obscure bacterial pathogens, will continue to yield important information on bacterial pathogenicity and immunobiology.

ACKNOWLEDGEMENTS

The Wellcome Trust and Medical Research Council have supported our work on flagella of Treponema and Helicobacter. We thank Theresa Klinowska for her contribution to electron microscopy of Treponema denticola flagella.

REFERENCES