10
Genetics and Assembly

Genetic Map

Some 50 genes encode products necessary for the assembly and operation of the chemotaxis system. These are shown on the genetic map of *E. coli* in Fig. 10.1. Arrows indicate operon structure, as described in the figure legend. Most of these genes specify components required for construction of the flagellar rotary motor. They fall into three hierarchical sets (Table 10.1). The early set specifies the transcriptional regulators, *FlhD* and *FlhC*, required for expression of all the other genes. The middle set encodes components of the hook-basal body, including the transport apparatus, rotor, drive shaft, bushing, hook, hook-associated proteins, and filament cap; recall Fig. 9.3. It also encodes a protein (FliA, alias σ^F or σ^{28}) destined to turn on the late genes, together with a protein, FlgM, that inactivates it. The regulatory proteins are listed in Table A.4 in the appendix. FlgM is pumped out of the cell by the transport apparatus when the hook-basal body is complete (Hughes et al., 1993; Kutsukake, 1994). This allows expression of genes that encode the filament (FliC), the force generators (MotA, MotB), and everything else required for direction control (receptors and *che*-gene products).

Essentially all of the genes and gene products required for bacterial chemotaxis are now known. Possible exceptions are genes (and gene products) required for essential cellular functions. Since a defect in such a gene blocks cell growth (is lethal), a more subtle approach is needed to learn whether it might be required for chemotaxis. The method of choice is isolation of conditional-lethal mutants, for example, mutants that are wild-type at one temperature but mutant at another temperature. Can cells be found that when grown at the permissive temperature and then switched to the nonpermissive one become nonchemotactic? Relatively little work of this kind has been done, because most, if not all, of the

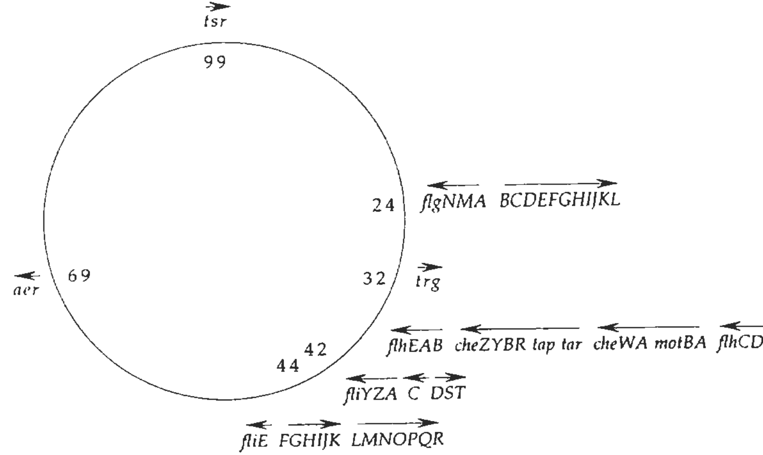

FIGURE 10.1. Genetic map of *E. coli* showing locations of the genes that make up the flagellar regulon. Arrows indicate operons (groups of genes transcribed on a single messenger RNA) and the direction of transcription. Transcription is initiated at a promoter, activated by a transcription factor; see Table 10.1. The genome is a closed circle of double-stranded DNA. It is calibrated in minutes, from 0 to 100, starting at the top. This scale arose from the time intervals required for the transfer of DNA from one cell to another during bacterial mating. The genes for many of the components of the basal body are located near 24 minutes; most of the others are between 42 and 44 minutes.

TABLE 10.1. Operon hierarchy for genes encoding proteins of *E. coli*'s chemotaxis system.

Early genes	Middle genes	Late genes
flhDC	*flgAMN*	*fliC*
	flgBCDEFGHIJKL	*motABcheAW*
	flhBAE	*tar tap cheRBYZ*
	fliAZY	*aer*
	fliDST	*trg*
	fliE	*tsr*
	fliFGHIJK	
	fliLMNOPQR	

Note: The genes that are underlined belong to the operons shown, activated by FlhDC, but they have additional promoters activated by FliA. Thus, they are expressed partially as middle genes and fully as late genes.

genes required for bacterial chemotaxis are not essential. Indeed, *E. coli* turns off the transcription (the reading) of its chemotaxis genes when grown in a suitably rich environment. And many laboratory strains of *E. coli* are nonmotile, because they have not had to compete for scarce nutrients. In short, if there is no need to hunt for food, then why bother to build the chemotaxis apparatus? Or if you try to make motors and something goes wrong, don't waste time and energy making filaments (an enormous undertaking).

The downregulation of the *flhDC* operon that results from growth on glucose (called catabolite repression) is well understood. It involves a cyclic adenosine monophosphate (cAMP) binding protein that interacts with DNA at a site upstream of *flhDC*. This protein activates *flhDC* and a number of other operons, but only when cAMP is present. The synthesis of cAMP is suppressed by a glucose catabolite. The upregulation of the *flhDC* operon that results from growth on a rich medium near a solid surface that leads to the hyperflagellation required for swarming (see Chapter 3) is poorly understood. *flhDC* is called the master operon. It is the target of a number of signals that gauge the state of the cell cycle and the external environment.

Flagellar Assembly

The motor is built from the inside out. Copies of FliF form the MS-ring, FliG,M,N the switch complex, and FlhA,B FliH,I,O,P,Q,R the transport apparatus (used for export of axial motor components); again, recall Fig. 9.3. Then copies of FlgB,C,F,G form the rod, FlgI the P-ring, FlgH the L-ring, and FlgE the hook. FliH and I are exported through the inner membrane by a different transport mechanism that involves cleavage of an N-terminal signal peptide. FliK is involved in switching export from FlgE to the hook-associated proteins, FlgK,L and FliD, and to the filament protein, FliC. FlgK,L and FliD assemble at the distal end of the hook, and FliC polymerizes under the FliD cap. The filament grows at its distal, not its proximal, end. Therefore, the flagellin subunits must pass through its axial pore. The FliD cap is essential for filament growth. If the cap is missing,

flagellin simply leaks out into the external medium. A cap rotation mechanism promotes filament assembly (Yonekura et al., 2000). Finally, MotA,B appear at the periphery of the MS-ring/switch complex, and the cell becomes motile.

Other components play accessory roles. FlgJ is a muramidase that cuts a hole through the peptidoglycan for the elongating rod, FlgA is a chaperone that assists in the assembly of FlgI into the P-ring, FlgD is a hook capping protein, and FlgN and FliS, T are chaperones that keep hook-associated and filament proteins unfolded until successfully transported. Control also occurs post-transcriptionally. For example, translation of the messenger RNA for the hook protein, FlgE, is suppressed after the transport apparatus is assembled but before the construction of the rod is complete. Mechanisms of this kind can regulate the synthesis of different proteins encoded at the same level of the transcriptional hierarchy.

The late genes also encode components required for control of the direction of flagellar rotation, that is, for chemoreception and signal processing (see Chapter 9).

For reviews on flagellar assembly and the control of flagllar gene expression, see Aizawa (1996), Chilcott and Hughes (2000), Aldridge and Hughes (2002), and Macnab (2003).

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