Identification and characterization of a gene that controls colony morphology and auto-aggregation in *Escherichia coli* K12

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Many *Escherichia coli* K12 strains undergo switching between two forms which differ in a number of surface properties including colony morphology and the ability to auto-aggregate. This paper describes the identification of a gene which appears to play a part in controlling this switching phenomenon. This gene has been designated *mor* and is located at 89 minutes on the *E. coli* chromosome map between the *argECBH* operon and the *trmA* gene. By manipulation of this gene it is possible to overcome the switching of surface properties and fix a strain in one form or the other. The *mor* gene has been cloned and its DNA sequence determined. The putative protein sequence shows a high level of homology with four regulatory genes, the *ilvY*, *cysB* and *lysR* genes from *E. coli* and the *metR* gene from *Salmonella typhimurium*. It has also been shown that the *mor* gene is autoregulated at the transcriptional level.

Introduction

Diderichsen (1980) described the existence in many *Escherichia coli* K12 strains of two interconvertible forms, which can be distinguished on the basis of colony morphology and their ability to form cell clumps in liquid media. One form, Form 1, is characterized by dull colonies with an irregular shape. This form also undergoes auto-aggregation in liquid culture and the cell clumps sediment to form a cell pellet when a shaken culture is left to stand. The other form, Form 2, is characterized by round, glossy colonies that tend to be smaller than the dull colonies. This form does not undergo auto-aggregation in liquid culture. Another feature which distinguishes the two forms is that Form 2 produces type 1 pili (fimbriae) on its surface, when grown in a static liquid culture, whereas Form 1 is not piliated. The rate of switching from Form 1 to Form 2 differs from the rate of switching from Form 2 to Form 1. When a colony in Form 1 is restreaked on an agar plate about 10% of colonies will have switched to Form 2.

When a colony in Form 2 is restreaked about 3% of colonies will have switched to Form 1.

Diderichsen (1980) identified a gene which is involved in controlling the switching of a strain between these two forms. He designated this gene *flu*, and mapped it to a position around 43 minutes on the *E. coli* chromosome. In addition to his work on the *flu* gene, Diderichsen also noted that when a segment in the 89 minute region of the chromosome is deleted from a strain exhibiting switching between Forms 1 and 2, this fixes the strain permanently in Form 1.

This paper describes work in which we have investigated further the genetic basis for the observation that a deletion in the 89 minute region can fix a strain in Form 1. This work has led to the characterization of a previously unidentified *E. coli* gene which we have designated *mor*. This gene is involved in the control of a number of surface properties, including colony morphology and auto-aggregation.

Methods

Bacterial strains and culture conditions. The *E. coli* K12 strains used in this study are shown in Table 1. LB broth and LB agar (Miller, 1972) were generally used for culture growth. Selection of auxotrophic markers was done using M9 medium (Miller, 1972) with appropriate supplements. Lac⁺ bacteria were detected using MacConkey agar

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Table 1. *Bacterial strains used in this work*

Strain	Genotype/phenotype	Source
BD1302	<i>metB</i> Δ (<i>ppc argECBH mor</i>) <i>relA spoT</i> (λ^+) <i>Hfr P4X</i>	B. Diderichsen, Novo Ltd, Denmark
BD1512	<i>lysA metE proB purE thi trp str supE tsx lac</i>	
JM108	<i>thi endA hsdR recA gyrA supE relA</i> Δ (<i>lac-proAB</i>)	
SW1007	<i>argE aroD his</i> Δ (<i>proA-gpt</i>) <i>thi shiA tsx gal mtl xyl recA</i> Tc ^R	R. Schmitt University of Regensburg, FRG
MC1022	<i>araD139</i> Δ (<i>ara,leu</i>)7697 Δ (<i>lacZ</i>)M15 <i>galU galK strA</i>	Constructed by transducing BD1712 (source: B. Diderichsen) with a <i>recA</i> mutation
N2026	<i>recB21 recC22 sbcB15 sbcC201 thi-1 proB48 his-4 argE3 lacI3 lacZ118 mtl-1 xyl-5 supE44 rpsL31</i>	C. Thomas University of Birmingham, UK
SW1066	<i>recB21 recC22 sbcB15 sbcC201 thi-1 proB48 his-4 argE3 lacI3 lacZ118 mtl-1 xyl-5 supE44 rpsL31 mor-lacZ</i> Km ^R	R. Lloyd University of Nottingham, UK
SW1099	<i>araD139</i> Δ (<i>ara,leu</i>)7697 Δ (<i>lacZ</i>)M15 <i>galU galK strA thi argE mor-lacZ</i> Km ^R	This work

(Oxoid). Ap^R, Tc^R, Cm^R and Km^R markers were selected using 50 µg ampicillin ml⁻¹, 20 µg tetracycline ml⁻¹, 25 µg chloramphenicol ml⁻¹ and 40 µg kanamycin ml⁻¹ respectively. All cultures were grown at 37 °C and liquid cultures were shaken at 200 r.p.m. in an orbital incubator.

P1 transduction. Strain construction was done by the method of Miller (1972).

Settling tests. The formation of cell aggregates was generally monitored by settling tests. Each test involved inoculating 5 ml of LB broth in a 30 ml plastic Universal bottle (Sterilin), incubating in an orbital shaker for 15 h and then leaving the bottle to stand. With a strain such as BD1302, which exhibits rapid sedimentation, up to 60% of the cells would have settled to form a distinct pellet within 1 h. Following prolonged standing (6 h), the medium above the pellet would be almost clear. The size of the cell aggregates which cause this sedimentation was measured using both laser light scattering (Malvern model 2600C) and a Coulter multisizer. The aggregates were between 3 and 6 µm across.

Inoculum-size-sensitivity. This was monitored by plating 3×10^6 cells on a LB-agar plate. A standard strain would give confluent growth, while an inoculum-size-sensitive strain would give small patches of growth and a few single colonies. If 3×10^4 cells were plated no growth would be obtained with an inoculum-size-sensitive strain.

Cosmid cloning and DNA manipulation. Cosmid cloning was done with the vector pEMBLcos4 using the method of Hadfield (1987). Chromosomal DNA was partially digested with *Sau3A* and the resulting fragments ligated with the cosmid arms formed by digesting pEMBLcos4 with *Bam*HI and *Pvu*II. The λ packaging mix used was supplied by Amersham. Plasmid DNA was generally prepared by the method of Birnboim & Doly (1979) although in some cases additional phenol extraction steps were done.

Transposon mutagenesis. The target plasmid (pSRW220 or pSRW226) was transformed into the strain JM108(pRU870) with selection for ampicillin and chloramphenicol resistance. Plasmid pRU870 contains the Cm^R transposon Tn1737Cm (Ubben & Schmitt, 1987) and is compatible with plasmids with pMB1-based replicons such as pSRW220 or pSRW226. Following transformation single colonies were left for approximately 2 weeks at room temperature. Whole colonies were then inoculated into 5 ml of LB broth, grown overnight

and plasmid DNA isolated from a 1 ml sample. Next a third of the DNA from each isolation was transformed into MC1022, and selection was done on LB agar containing ampicillin and chloramphenicol. One transformant from each transformation was then taken and plasmid DNA purified. At this stage a check was made to ensure that an insertion derivative of the target plasmid had been isolated and that there had not been a double transformation resulting, for example, in the presence of unmodified plasmid alongside the insertion derivative. In each case the position of the transposon insertion was determined to an accuracy of about 100 bp by restriction mapping.

In Tn1737Cm the *lacZ* coding sequence is located at one end (Ubben & Schmitt, 1987). There is no promoter for *lacZ* within the transposon and so β -galactosidase is only produced if the transposon becomes inserted downstream from a promoter in the correct orientation. The formation of transcriptional fusions with *lacZ* was detected using MacConkey agar plates.

DNA sequencing. This was done using the methods described by Varley & Boulnois (1984) and Walker *et al.* (1987). Both strands were sequenced.

Integration of linear DNA fragments into the chromosome. This was done as described by Jasin & Schimmel (1984).

Determination of β -galactosidase activity. The method of Miller (1972) was used; activities were calculated in arbitrary units.

Results

Cloning of a gene from the 89 minute region of the E. coli chromosome which is involved in controlling colony morphology and auto-aggregation

The deletion in strain BD1302 (Diderichsen, 1980) that causes it to be fixed in Form 1 removes a segment of DNA that includes the *argECBH* operon. Therefore, in order to isolate DNA from this region of the chromosome, cosmid cloning was done using the Arg⁻ strain SW1007 as the recipient and initial selection was for

Fig. 1. Structure of the plasmids pSRW220 and pSRW226. ■, Chromosomally derived DNA; —, pBR322-derived DNA. E, B, Bg, P and S represent the sites for the restriction enzymes *EcoRI*, *BamHI*, *BglII*, *PstI* and *SalI* respectively. Ap^R represents the gene for ampicillin resistance.

Arg⁺ clones. The chromosomal DNA used for this cosmid bank was isolated from strain BD1512 which undergoes switching between Forms 1 and 2. Cosmid DNA from one of the Arg⁺ clones that was obtained was then cut with *HindIII* and *SalI* and the fragments produced were ligated with pBR322 DNA that had been cut with *HindIII* and *SalI*. The resulting plasmids were then screened for the ability to complement the genetic defect in strain BD1302 that causes it to be fixed in Form 1. This was done by transforming the plasmids into BD1302 and by using settling tests to identify plasmids capable of preventing the rapid sedimentation of cells from a static suspension that is characteristic of this strain. One such plasmid was identified and designated pSRW220 (Fig. 1). In settling tests with BD1302(pSRW220) all of the cells remained in suspension after 1 h in contrast to the situation with BD1302 where up to 60% of the cells sedimented within this time.

pSRW220 contained a 6.0 kb insert of chromosomal DNA (Fig. 1). Comparison was made with previous studies in which restriction mapping of the chromosomal region including *argECBH* and *trmA* was done (Ny & Björk, 1980; Heller *et al.*, 1985). From these data it was concluded that the 6.0 kb fragment in pSRW220 contained the 3'-end of the *argCBH* transcription unit, the 3'-end of the *trmA* gene and the whole of the region between these two loci.

Further subcloning from pSRW220 revealed that when the 2.3 kb *EcoRI*–*BamHI* fragment from pSRW220 was inserted between the *EcoRI* and *BamHI* sites of pBR322 to form pSRW226 (Fig. 1), this smaller plasmid also complemented the genetic defect in strain BD1302 as judged by its ability to prevent the rapid sedimentation of cells from a static suspension. Close examination of the colony morphology of BD1302(pSRW226) revealed that all the colonies were

Fig. 2. Location and effect of Tn1737Cm insertions into the chromosomally derived DNA of pSRW220 and pSRW226. Those insertions which caused insertional inactivation of the *mor* gene are marked with an X. The orientation of each insert is shown by indicating the position of the *lacZ* coding sequence in each case. Those insertion derivatives in which the *lacZ* sequence was transcribed as judged by the observation of a red colouration on MacConkey agar are marked with a +. Those insertion derivatives in which there was no detectable transcription of the *lacZ* gene are marked with a -. B, E and S indicate the sites for the restriction enzymes *BamHI*, *EcoRI* and *SalI* respectively.

glossy and that no dull colonies were produced. It thus became apparent that this strain does not undergo switching and is fixed in Form 2. Furthermore the introduction of pSRW226 into BD1302 was found to overcome the inoculum-size-sensitivity of this strain.

Identification of the *mor* gene by transposon mutagenesis

To locate more accurately the position of the gene which had been cloned, transposon mutagenesis of both pSRW220 and pSRW226 was done using Tn1737Cm (Ubben & Schmitt, 1987). The results of this transposon mutagenesis are shown in Fig. 2. Insertion derivatives pSRW226.1, 226.3, 226.5, 226.6 and 220.1 all exhibited insertional inactivation and failed to complement the deletion in BD1302. This was shown by the observation of rapid sedimentation of cells in settling tests. Further checks on colony morphology revealed that the dull phenotype was exhibited. The positions at which insertion had occurred were taken to define the position of the gene whose introduction into BD1302 on a plasmid fixes the strain in Form 2. This gene was designated *mor* (colony morphology). A transcriptional analysis of this region was made possible by the presence of the *lacZ* coding sequence at one end of Tn1737Cm. Two interesting points emerged from testing the different insertion derivatives for expression of β -galactosidase. First, the detection of β -galactosidase activity with pSRW226.3, 226.1 and 220.1 indicated that the direction

1	GGATCCTGGA	GATCCGCAA	AGTTCACGTT	GGCTTTAGTT	ATTCGAGTTG
51	AGAAACTCTC	GAAACGGGCA	GTGACTTCAA	GGGTAAAAAG	AGGTGCCCGT
101	CCGTTTCTGT	GAGCAATTAT	CAGTCAGAAT	⁻³⁵ GCTTGATAGG	GATAATCGTT
151	⁻¹⁰ CATTGCTATT	CTACCTATCG	CCATGAACTA	TCGTGGCGAT	^{S D} GGAGGATGGA
201	TAATGAATAT	TCGTGATCTT	GAGTACCTGG	TGGCATTGGC	TGAACACCGC
251	CATTTTCGGC	GTGCGGCAGA	TTCCTGCCAC	GTTAGCCAGC	CGACGCTTAG
301	CGGGCAAATT	CGTAAGCTGG	AAGATGAGCT	GGGCGTGATG	TTGCTGGAGC
351	GGACCAGCCG	TAAAGTGTG	TTCACCCAGG	CGGGAATGCT	GCTGGTGGAT
401	CAGGCGCGTA	CCGTGCTGCG	TGAGGTGAAA	GTCCTTAAAG	AGATGGCAAG
451	CCAGCAGGGC	GAGACGATGT	CCGGACCGCT	GCACATTGGT	TTGATTCCCA
501	CAGTTGGACC	GTACCTGCTA	CCGCATATTA	TCCCTATGCT	GCACCAGACC
551	TTTCCAAAGC	TGGAATGTGA	TCTGCATGAA	GCACAGACCC	ACCAGTTACT
601	GGCGCAACTG	GACAGCGGCA	AACTCGATTG	CGTGATCCTC	GCGCTGGTGA
651	AAGAGAGCGA	AGCATTCAAT	GAAGTGCCGT	TGTTTGATGA	GCCAATGTTG
701	CTGGCTATCT	ATGAAGATCA	CCCGTGGGCG	AACCGCGAAT	GCGTACCGAT
751	GGCCGATCTG	GCAGGGGAAA	AACTGCTGAT	GCTGGAAGAT	GGTCACTGTT
801	TGCGCGATCA	GGCAATGGGT	TTCTGTTTTG	AAGCCGGGGC	GGATGAAGAT
851	ACACACTTCC	GCGCGACCAG	CCTGGAAGCT	CTGCGCAACA	TGGTGGCGGC
901	AGGTAGCGGG	ATCACTTTAC	TGCCAGCGCT	GGCTGTGCCG	CCGGAGGCCA
951	AACGCGATGG	GTTTGTAT	CTGCCGTGCA	TTAAGCCGGA	ACCACGCCGC
1001	ACTATTGGCC	TGGTTTATCG	TCCTGGCTCA	CCGCTGCGCA	GCCGCTATGA
1051	GCAGCTGGCA	GAGGCCATCC	GCGCAAGAAT	GGATGGCCAT	TTCGATAAAG
1101	TTTTAAACA	GGCGTTTAA	ACCGTTTAAC	¹ GCAGCTACCC	GATAGCTTCG
1151	CCATCGTCGG	¹ GTAGTTAAAG	² GTGGTGTTGA	CGAAGTACTC	AATAGTGTTC
1201	² CCGCCACCTT	TCTGTTCCAT	AATCGCCTGA	CCGATATGAA	TAATTTCCGC
1251	AGCGCGCTCG	CCAAAGCAGT	GAATACCCAG	AATCTCTTTT	GTTTCCCGAT
1301	GGAACAAAAT	TTTCAGCGTG	CCCACGTTCA	TGCCGACGAT	TTGTGCGCGT
1351	GCCAGATGTT	TAAACTGGGC	GCGGCCCACT	TCATATGGCA	CTTTCATTGC
1401	GGTCAGCTGC	TGTTCGGTTT	TGCCACAGAG	GCTGATTTCC	GGGATGGTGT
1451	AAATACCGGT	AGGGATATCT	TCAATCAGAT	GTGCGGTGGC	TTGCGCTTTT

of transcription of the *mor* gene is away from the *Bam*HI site of pSRW226 towards the *Eco*RI site. Second, the failure to detect transcription of β -galactosidase with pSRW226.6, even though the insert in this plasmid is in the same orientation as the insert in pSRW226.3, suggested that in the former plasmid insertional inactivation in the region of the *mor* promoter has occurred.

Sequence of the *mor* gene

The sequence of the 2.3 kb *Bam*HI–*Eco*RI fragment of pSRW226 was determined. Fig. 3 shows the first 1.5 kb of this sequence starting from the *Bam*HI site. The results obtained from the transposon mutagenesis enabled the *mor* gene to be readily identified. The region delimited by the transposon inserts, which brought about insertional inactivation, contained an open reading frame capable of encoding a 305-amino-acid protein. Immediately upstream from this coding sequence was a region showing all the characteristics of an *E. coli* translation initiation site (Kozak, 1983). Moreover, a sequence showing strong homology to the consensus *E. coli* promoter sequence (Hawley & McClure, 1983) was identified in the region where the *mor* gene promoter was predicted to lie following the results obtained with insertion derivative pSRW226.6.

The Swiss-Prot protein sequence data base was screened using the FASTP method of computer searching (Lipman & Pearson, 1985) for sequences showing homology to the deduced amino acid sequence of the *mor* gene product. Four proteins showing a high level of homology were identified. These were the *ilvY*, *cysB* and *lysR* proteins of *E. coli* (Wek & Hatfield, 1986; Ostrowski *et al.*, 1987; Stragier & Pate, 1983) and the *metR* protein of *Salmonella typhimurium* (Plamann & Stauffer, 1987). The percentage homologies that the putative *mor* gene product exhibits with these four proteins are listed in Table 2. It can be seen that *ilvY* shows the highest homology with 29.4% identity in a 262 amino acid overlap. This homology is illustrated in Fig. 4.

Demonstration that the *mor* gene is autoregulated

The observation of homology between the putative *mor* gene product and the regulatory proteins *ilvY*, *cysB*, *lysR* and *metR* led to the tentative proposal that the *mor* protein might have a regulatory function. In order to

Table 2. Percentage identity between various members of the *mor*, *ilvY*, *lysR*, *cysB* and *metR* protein family

	Percentage identity*				
	<i>mor</i>	<i>ilvY</i>	<i>lysR</i>	<i>cysB</i>	<i>metR</i>
<i>mor</i>	100	29.4 (262)	20.7 (305)	26.0 (288)	26.7 (255)
<i>ilvY</i>	—	100	21.6 (292)	21.4 (262)	26.6 (252)
<i>lysR</i>	—	—	100	22.2 (257)	23.0 (257)
<i>cysB</i>	—	—	—	100	22.2 (257)
<i>metR</i>	—	—	—	—	100

* The length of the amino acid overlap to which each percentage identity refers is shown in parentheses.

obtain further evidence for such a proposal, it was decided to investigate whether expression of the *mor* gene is autoregulated. Such a regulatory mechanism has been shown to operate in the case of a number of previously characterized regulatory genes, including *lysR* (Stragier & Pate, 1983) and *cysB* (Ostrowski *et al.*, 1987). In order to test for autoregulation use was made of the transcriptional fusion in pSRW220.1 (see above) which has Tn1737Cm inserted in such a way that the *lacZ* coding sequence is transcribed from the *mor* promoter. Further transposition of Tn1737Cm was prevented by removing the *tnpR* and *tnpA* transposition functions from pSRW220.1 and replacing them with the kanamycin-resistance gene of Tn5. The resulting plasmid was designated pSRW231 and its construction is illustrated in Fig. 5. The *mor*–*lacZ* fusion was then inserted into the chromosome by cutting pSRW231 with the restriction enzyme *Tth*111I and transforming the linear DNA into N2026 with selection for kanamycin resistance. Integration of the *mor*–*lacZ* fusion into the chromosome was thus allowed to take place by homologous recombination of the DNA flanking the transcriptional fusion with the corresponding DNA in the chromosome. The strain formed in this way was designated SW1066. The *mor*–*lacZ* fusion was then transferred into the *lac* strain MC1022 by P1 transduction with selection for kanamycin resistance and the strain so formed was designated SW1099.

To test for autoregulation of the *mor* promoter, the level of β -galactosidase expression from the *mor*–*lacZ* fusion was compared in the following three strains, SW1099(pSRW226), SW1099(pKO6) and SW1099. Plasmid pKO6 (McKenney *et al.*, 1981) was used as a

Fig. 3. Sequence of the chromosomal region which contains the *mor* gene. The sequence shown starts at the *Bam*HI site of pSRW226. The –10 and –35 boxes of the putative *mor* promoter are underlined as is the putative Shine–Dalgarno sequence (S–D). The open reading frame, which from the transposon mutagenesis would appear to be the *mor* coding sequence, lies between a start codon at position 203 and a stop codon at position 1118. Two inverted repeat sequences at the 3'-end of the *mor* gene are marked by arrows and are labelled 1 and 2.

Fig. 4. Homology between the predicted *mor* amino acid sequence and the *ilvY* protein. Upper line, predicted amino acid sequence of *mor* protein; lower line, amino acid sequence of *ilvY* protein. Pairs of dots between the lines indicate positions with the same amino acid in both sequences. Single connecting dots indicate amino acid substitutions which occur most frequently in evolution. A sequence in the putative *mor* polypeptide with potential for forming a helix-turn-helix DNA binding motif (see Discussion) is indicated with two vertical lines.

control for comparison with pSRW226 because it has the same replicon and antibiotic resistance marker, but does not contain the *mor* gene. A marked difference in the level of expression from the chromosomal *mor-lacZ* fusion was observed depending on whether or not the *mor*-containing plasmid pSRW226 was present. Both SW1099(pKO6) and SW1099 exhibited similar β -galactosidase activities (538 ± 139 and 589 ± 121 arbitrary units respectively), while SW1099(pSRW226) exhibited a greatly reduced activity (32 ± 7). Thus, the presence of the *mor* gene was shown to bring about a 17- to 18-fold repression of the *mor-lacZ* fusion, clearly indicating that the *mor* gene is autoregulated.

Discussion

The work described in this paper indicates that by manipulation of the *mor* gene it is possible to overcome the switching of an *E. coli* K12 strain between two forms with differing surface properties. It has been demonstrated that when a plasmid containing the *mor* gene, such as pSRW226, is introduced into a strain, this can result in it becoming fixed in a form exhibiting a glossy colony morphology and which does not undergo auto-aggregation (Form 2). Alternatively, inactivation of the *mor* gene can fix a strain in Form 1. It should be borne in mind, however, that the phenotypes described in this

Fig. 5. Construction of pSRW231. ■, Chromosomally derived DNA; □, Tn1737Cm-derived DNA; ▨, pUC4-K1XX-derived DNA (Barany, 1985); —, pBR322-derived DNA. St and T represent the sites for the restriction enzymes *StuI* and *Tth1111*. *Cm^r* and *Km^r* represent genes for chloramphenicol and kanamycin resistance. The resolvase and transposase genes of Tn1737Cm are represented by *tnpR* and *tnpA* respectively. During the construction of pSRW231 all of the *tnpR* coding sequence is removed along with about 450 amino acids from the N-terminus of *tnpA* (Brown *et al.*, 1985).

paper correspond to observations made with only one particular strain, BD1302. Preliminary experiments to determine the effect of inactivating *mor* gene activity in other *E. coli* strains have been done: these have shown that the phenotype associated with a *mor* mutation is dependent upon the genetic background into which it is introduced.

The mechanism by which pSRW226 can fix a strain in Form 2 is as yet unknown. One possibility is that expression of the *mor* gene is continually switching on and off as the result of a DNA inversion event similar to that which controls expression of the pilin gene (*fimA*) in *E. coli* (Abraham *et al.*, 1985). If this were the case the introduction of multiple copies of the *mor* gene on a plasmid would result in there being a very high probability that at least one copy of the gene would be in the active form at any given time. However no evidence for such switching was obtained using the *mor-lacZ* fusion. Further work is necessary to test this more

rigorously. A second possibility is that there is another gene on the chromosome which is actually switching on and off and that the *mor* gene is involved in controlling this second gene in some way. For example, the *mor* gene could stimulate the switching of this second gene in one particular direction (either from off to on or from on to off). This latter mechanism would be similar to the action of the *fimB* and *fimE* regulatory genes which control the *fimA* gene (Klemm, 1986).

The results obtained suggest that the *mor* gene exerts its control over surface properties by encoding a regulatory protein rather than by directly encoding a surface component. The evidence for this comes from two lines of work. First, the sequence of the putative *mor* gene product shows a high level of homology to four regulatory proteins, *ilvY*, *cysB*, *lysR* and *metR*. Second, the *mor* gene is autoregulated and transcription from the *mor* promoter is repressed in the presence of excess *mor* gene product. In view of this evidence for a regulatory role, the putative *mor* protein sequence was searched using the method of Dodd & Egan (1987) for sequences with potential for forming a helix-turn-helix DNA binding motif (Pabo & Sauer, 1984). This analysis revealed a 20-amino-acid sequence with a favourable score of 1523 (a score of over 1000 is taken to indicate a sequence with a potentially significant similarity to the helix-turn-helix motif). This sequence is located between residues 17 and 38 of the predicted *mor* protein sequence.

Another point worth noting is that all four of the proteins showing a high level of homology to the putative *mor* gene product are positive regulators of gene expression. It is therefore interesting to speculate that, in addition to acting as a negative regulator of its own expression, the *mor* gene product may exert its influence over cell surface properties by acting as a positive regulator.

The demonstration that the manipulation of the *mor* gene can affect both colony morphology and auto-aggregation suggests that it must exert control over the production of one or more surface components. One surface component which would at first sight seem a prime candidate to explain the molecular basis of the surface properties observed is the lipopolysaccharide (LPS) component of the outer membrane. Wild-type *E. coli* can exist in two forms, known as S and R, with different LPS structures. The results described in this paper cannot, however, be explained in terms of the existence of these S and R forms. This is because all of the strains which have been used in this study are derivatives of *E. coli* K12; this carries a *rfb* mutation (Schmidt, 1973) which results in it permanently being in the R form. Thus it remains uncertain which surface components are responsible for the phenotypes described in this paper.

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