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Virulence gene regulation in pathogenic *Escherichia coli*

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Abstract – The ability to regulate gene expression throughout the course of an infection is important for the survival of a pathogen in the host. Thus, virulence gene expression responds to environmental signals in many complex ways. Frequently, global regulatory factors associated with specific regulators co-ordinate expression of virulence genes. In this review, we present well-described regulatory mechanisms used to co-ordinate the expression of virulence factors by pathogenic *Escherichia coli* with a relative emphasis on diseases caused by *E. coli* in animals. Many of the virulence-associated genes of pathogenic *E. coli* respond to environmental conditions. The involvement of global regulators, including housekeeping regulons and virulence regulons, specific regulators and then sensor regulatory systems involved in virulence, is described. Specific regulation mechanisms are illustrated using the regulation of genes encoding for fimbriae, curli, haemolysin and capsules as examples. © Inra/Elsevier, Paris.

Escherichia coli / virulence gene / regulation

Résumé – Régulation de l'expression des gènes de virulence chez les *Escherichia coli* pathogènes. Le contrôle de l'expression génétique d'un pathogène au cours d'une infection s'avère une propriété importante pour sa survie dans l'hôte. Ainsi, des signaux environnementaux influencent, par diverses voies, l'expression des gènes de virulence. Cette dernière est coordonnée par des facteurs de régulation généraux, lesquels sont souvent en association avec des éléments régulateurs spécifiques. Dans cette revue, des mécanismes de régulation bien étudiés impliqués dans l'expression des facteurs de virulence chez les *Escherichia coli* pathogènes sont présentés, une certaine emphase étant mise sur les *E. coli* responsables des maladies animales. Chez les *E. coli* pathogènes, l'expression de plusieurs gènes codant pour des déterminants de virulence est soumise aux conditions du milieu environnant. La régulation de l'expression des facteurs de virulence des *E. coli* pathogènes par des régulateurs globaux, lesquels incluent les régulons de gènes de ménage de même que des régulons de virulence, par

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des régulateurs spécifiques ainsi que par des systèmes régulateurs réagissant à la détection d'un signal, sera présentée. Les mécanismes spécifiques de régulation seront illustrés en présentant, entre autres, des systèmes de régulation génétique concernant l'expression des fimbriae, de curli, de l'hémolysine et des capsules. © Inra/Elsevier, Paris.

***Escherichia coli* / gène de virulence / régulation**

Plan

1. Introduction.....	132
2. Environmental regulation of virulence gene expression.....	133
3. Global regulators of gene expression.....	134
3.1. H-NS.....	135
3.1.1. Regulatory role of H-NS in fimbrial gene expression.....	135
3.1.2. Regulatory role of H-NS in enteroinvasive <i>E. coli</i> (EIEC) virulence gene expression.....	136
3.1.3. Effect of H-NS on haemolysin production.....	137
3.1.4. Effect of <i>typA</i> on H-NS expression.....	137
3.2. IHF.....	138
3.3. CRP.....	138
3.4. RpoS.....	138
3.5. Lrp.....	138
3.6. Fur.....	139
4. Family of the AraC transcriptional activator.....	139
5. Sensor regulatory processes.....	140
6. tRNA regulation of virulence genes.....	141
7. Black holes and virulence gene expression.....	142
8. Regulation of fimbrial expression.....	142
8.1. The <i>pap</i> regulatory family.....	142
8.2. Regulation of type I fimbriae expression.....	145
8.3. Regulation of K99 expression.....	145
8.4. Regulation of curli expression.....	146
9. Regulation of haemolysin expression.....	146
9.1. DNA supercoiling.....	147
9.2. Transcription elongation.....	147
9.3. Stabilization of the active conformation of the toxin.....	148
10. Regulation of capsule synthesis.....	148
11. Perspectives.....	149

1. INTRODUCTION

During the course of infection, pathogenic micro-organisms encounter different types of environments and they have to adapt in order to survive and multiply. Thus, pathogenic bacteria need to synthesize virulence factors that will allow them to colonize a hostile environment and to survive the host immune and non-immune defences. However, the expression of a virulence factor is not advantageous to the bac-

terial survival during all the infectious process. For example, an adhesin can be advantageous for the adhesion of a bacteria on intestinal mucosa but becomes disadvantageous when the bacteria is in the bloodstream or when the bacteria needs to escape the bactericidal activity of serum. Therefore, micro-organisms sense the environment and, in response to the signals that they receive, act accordingly by turning off or on the expression of their virulence genes [40, 51]. For the majority of the virulence

factors, the specific host signals that the regulatory protein detects are not yet understood, although the environmental signals that modulate the expression of virulence genes have in many cases been identified *in vitro*. Parameters such as temperature, osmolarity, pH, source of nitrogen, concentrations of iron, sugars and amino acids are known to affect the regulation of virulence genes *in vitro*. One challenge of the next few years will be to find out if these parameters play a role in virulence factor expression *in vivo*.

The regulation of pathogenicity is complex. There are interconnections between regulatory systems. A single regulator can control the expression of several genes (global regulator) including virulence genes and housekeeping genes. For example, the leucine responsive protein (Lrp), the cAMP receptor protein (CRP), the histone-like protein (H-NS) and the sigma factor RpoS are global regulators that are involved in regulatory networks that control virulence genes, genes encoding metabolic enzymes and structural components. Often, global and specific regulators simultaneously affect virulence gene expression which is optimally co-ordinated to permit the survival of a pathogen in a particular ecological niche.

Here, we review the well-described regulatory mechanisms used to co-ordinate the expression of virulence factors by pathogenic *E. coli* with a relative emphasis on *E. coli* causing diseases in animals. Some regulatory mechanisms that have been described for other pathogenic bacteria might also exist in pathogenic *E. coli* but are not evoked here owing to the lack of experimental results in *E. coli*. The regulation of expression of virulence determinants by global regulators, including housekeeping regulons and virulence regulons, specific regulators and then two component regulatory systems involved in virulence, is presented. Specific regulation mechanisms are illustrated using the regulation of genes encoding fimbriae, curli, haemolysin or capsules as examples.

2. ENVIRONMENTAL REGULATION OF VIRULENCE GENE EXPRESSION

Not all virulence factors confer a selective advantage to the microbe at the same stage of infection or at the same anatomical site within the host. Consequently, the expression of certain virulence factors must be modulated in response to environmental signals encountered throughout the infectious cycle and during the transition from the external milieu to the host. Such regulation allows for the co-ordinated expression of proteins required for survival in different environmental niches. Virulence factors, such as adhesins, toxins, siderophores, etc., must be synthesized only in the appropriate location in the host so that bacteria can multiply, colonize host tissues and evade the host immune response.

In vivo, bacteria first need to sense that they have entered into a host. Temperature is an appropriate signal since the temperature of mammalian bodies is generally around 37 °C, higher than the external environment. Thus, several virulence factors such as adhesins, haemolysin, or enteropathogenic *E. coli* (EPEC)-, RDEC-1 (a rabbit EPEC strain)- and STEC (shiga-like toxin producing *E. coli*)-secreted proteins are specifically expressed at the normal host body temperature [1, 34, 71, 78, 89]. Iron availability is also an indicator of the host environment since iron is sequestered in the body by specific proteins such as transferrin or lactoferrin. Thus, limiting iron concentrations often induce the expression of virulence factors, as is the case for siderophores, shiga-like toxins or haemolysin [18, 49, 75].

Second, bacteria need to sense which organ they have reached. Bacteria travelling through the gastrointestinal lumen of omnivorous mammals are subjected to a low pH stress in the stomach, followed by a more hospitable environment with respect to pH in the intestinal lumen varying from pH 6.5 to 7.5 [38]. The regulation of gene expression in response to changes in the pH

is complex and is associated with pH variations, ion concentration, proton-motive force and membrane potential [26, 100]. Acid tolerance is highly dependent on the growth phase. Maximal acid resistance of the *E. coli* strain MC4100 is exhibited at the stationary growth phase and is dependent on RpoS, although RpoS is not an absolute requirement under all growth conditions [122]. Expression of several adhesins and EPEC-secreted proteins is inhibited at low or high pH. By using promoter fusions to measure the response according to changes in pH, it was observed that the expression of fimbrial genes such as *fas* (987P) and *foo* (F165₁) responded to changes in pH [36]. High concentrations of monoamines, most notably norepinephrine, may be one of the signals of the small intestine environment. Indeed, norepinephrine can increase the growth and production of virulence factors of ETEC (K99) and EHEC (SLT-I, SLT-II), and this effect is non-nutritional in nature [81]. Osmolarity, carbon source, concentration of O₂, ammonia, sodium bicarbonate and amino acids can combine to precisely indicate the location of the bacteria inside an organ [35]. As an example, Edwards et al. [36] proposed that carbon and/or nitrogen gradients in the gut provide a mechanism that allows preferential colonization of different segments by various enteropathogens. Glucose concentrations are higher in the proximal small intestine than in the distal portion, in contrast to nitrogen concentrations which are higher in the distal segment. The porcine 987P ETEC adhesin is maximally expressed in conditions of limiting carbon and in the presence of ammonium, whereas the expression of the bundle forming pili (Bfp) EPEC adhesin is optimal during growth with glucose as a carbon source and is repressed by ammonium [110, 35]. Consequently, 987P fimbriated *E. coli* will colonize the distal small intestine. If Bfp expression is activated at an early stage of infection, Bfp fimbriated EPEC will colonize proximal as well as distant segments of the small intestine. The high concentration

of ammonium in the colon might, however, inform EPEC that it is not in an environment appropriate for survival, and thus adhesin expression is suppressed.

Effects of the environmental factors described above have been studied *in vitro*. Very few studies have been reported concerning the regulation of *E. coli* virulence factors expression *in vivo*, i.e. in the animal. The question is: are the regulations depicted *in vitro* indeed occurring in the host? Experimental infections followed by the detection of virulence factor expression in the organs or tissues are necessary to answer this concern. Pourbakhsh et al. [109] inoculated chicken air sacs with septicemic avian *E. coli* isolates and showed that a much higher proportion of bacteria colonizing the trachea were F1 (type I fimbriae) fimbriated as compared to bacteria colonizing the lungs, air sacs and systemic organs, suggesting that the bacteria undergo a phase variation with respect to F1 fimbriae *in vivo*. A similar F1 fimbrial phase variation also occurs *in vivo* during the experimental induction of *E. coli* peritonitis and lower urinary tract infection in mice [4, 62, 97] and meningitis in rats [116]. In the same study Pourbakhsh et al. showed that P fimbriae are expressed in bacteria present in air sacs and systemic organs, but not in trachea, suggesting that P fimbriae also undergo phase variation *in vivo* [109]. The nature of the signal controlling the expression of these fimbriae is, however, unknown. The aim of the next few years will be to identify the nature of the signals that work *in vivo*, the sensing molecules involved, the mechanism of signal transduction, and to determine their activities on virulence factors in a given location within the host.

3. GLOBAL REGULATORS OF GENE EXPRESSION

Global regulators are involved in the regulation of virulence gene expression. In pathogenic bacteria, H-NS, CRP, RpoS, Lrp, play a role in sorting out complex signals

that modulate the synthesis of virulence factors. Some of them, such as CRP, recognize specific nucleotide sequences to which they bind. Their effects are confined to those genes possessing these specific binding sites. Others, such as H-NS, have a wider influence in controlling transcription and contribute to the organization of DNA topology in the cell. It has been shown that levels of supercoiling vary in response to environmental changes; in particular, growth of bacterial cells under anaerobic conditions, at a high osmolarity, at different temperatures, or in a nutrient-poor medium [29]. Topoisomerases are enzymes that act upon DNA to alter the level of supercoiling, as well as catenate and decatenate chromosomes. In the bacterial cell, DNA is negatively supercoiled [33]. It is thought that negative superhelical tension facilitates the melting of DNA necessary for replication and transcription [79]. This mechanism may be used by pathogenic *E. coli* to regulate genes necessary for virulence.

E. coli possesses low molecular weight proteins which contribute to the higher order organization of the bacterial nucleoid and to the expression of the genetic information. Frequently, small architectural proteins such as protein HU (a histone-like protein), the related protein IHF (integration host factor), H-NS and FIS (factor for inversion stimulation) contribute to the control of transcription of genes whose products play a role in environmental adaptation and thus in the expression of the virulence.

3.1. H-NS

H-NS is one of the two most abundant histone-like proteins in *E. coli* (the first one being HU). It is a 16-kDa neutral protein present at 20 000 copies per cell under a dimeric form (for a review, see [137]). H-NS can affect the overall structure of DNA by strongly compacting DNA, altering DNA superhelicity by introducing negative supercoils, and by the induction of DNA bending. H-NS appears, however, to play not

only a structural role in the organization of the chromosome, but also a rather dynamic role in the regulation of gene expression [56, 63, 129]. Many of the genes regulated by H-NS are involved in bacterial adaptation to environmental stress in response to signals such as osmolarity, temperature and oxygen availability [6]. Transcription of many virulence genes in *E. coli* is repressed by H-NS, and different transcriptional regulators act on gene expression by alleviating and/or counteracting the effect of H-NS. In some cases, H-NS seems to be implicated in the thermoregulation of virulence factor expression. The mechanisms by which H-NS affects gene transcription are not completely understood. Correlation between modifications of the overall DNA supercoiling induced by H-NS and gene expression is not clear. In some cases, H-NS can bind DNA to directly inhibit gene transcription [27, 129], and it has been suggested that H-NS prevents the RNA polymerase from productively interacting with the promoter [129]. Göransson et al. [47] have proposed that H-NS could act as 'silencers' of DNA regions by forming nucleoprotein structures similar to transcriptionally inactive chromatin. However, these effects are not indiscriminate as not all promoters are H-NS repressible. H-NS also participates in the control of sigma S synthesis [10]. Several examples of such regulations involved in fimbriae and haemolysin synthesis as well as in bacterial invasiveness are given below.

3.1.1. Regulatory role of H-NS in fimbrial gene expression

The *sfa* determinant codes for S fimbrial adhesins of extraintestinal *E. coli*. SfaA is the major structural subunit, whereas SfaB and SfaC are positive regulators of *sfa* transcription. Most *sfa* transcription initiates at the *sfaB* promoter, the *sfaA* promoter being very poorly active. Frameshift mutations in *sfaB* or *sfaC* result in very low levels of *sfa* transcription. Expression of S fimbriae from *sfaB/sfaC* mutants is, however, restored in

hns mutants, where pA transcription is strongly activated [87]. The authors conclude that the negative control exerted by H-NS on *sfa* transcription is counteracted by the *sfa*-specific activators SfaB and SfaC (figure 1).

P fimbriae are produced by cells growing at 37 °C but not at lower temperatures such as 25 °C. H-NS plays an important role in this thermoregulation by repressing the transcription of a regulatory cistron in the *pap* gene cluster. PapB and PapI are positive regulators homologous to SfaB and SfaC, respectively. PapI transcription is derepressed in *hns* mutants at both 37 and 26 °C. Transcription of the *papBA* operon is also derepressed at both temperatures although to a lesser extent [47, 136]. By competitive gel retardation assays it was shown that H-NS specifically binds to *pap* DNA containing *pap* GATC sites and was able to block methylation of these sites *in vitro*. It was suggested that the ability of H-NS to act as a methylation blocking factor is dependent upon the formation of a specific complex of H-NS with *pap* regulatory DNA. Transcription of *pap* is enhanced by binding of the cAMP-CRP complex in the intergenic *papI-papB* DNA. In *hns* mutants, the *pap* operon is expressed in the absence of the PapB and cAMP-CRP transcriptional activators. It has been suggested that the cAMP-CRP complex would play a role as an anti-repressor alleviating the H-NS-mediated silencing [41].

H-NS is also involved in the phase variation control of type 1 fimbriae [27, 30, 101]. FimB and FimE are recombinases that promote the inversion of a 314-bp DNA segment containing the *fimA* promoter. FimA is the major structural subunit of type 1 fimbriae. In *hns* mutants, transcription of *fimB* and *fimE* is increased at 30 °C and to a lesser extent at 37 °C, leading to an increased inversion rate of the *fimA* promoter. Donato et al. [27] have shown that H-NS specifically and co-operatively binds to the *fimB* promoter region and represses transcription. They propose that H-NS recognizes a specific DNA feature rather than

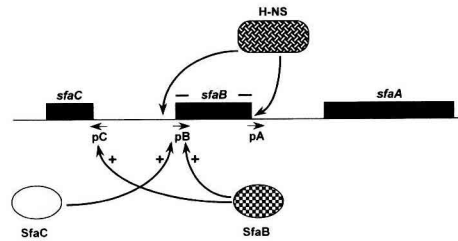


Figure 1. Model of the transcriptional regulation of the *sfa* genes. The *sfa* genes are under the negative control of the H-NS protein, which is counteracted by the fimbriae-specific activators SfaB and SfaC. *p*, promoter; -, negative action; +, positive action (from [86] with permission).

a specific consensus sequence. This feature could be a specific conformation such as an intrinsic curvature due to the known affinity of H-NS for curved DNA [137]. This was recently confirmed by competitive gel retardation assays where it was shown that H-NS binds to the regions containing *fimB* and *fimE* promoters and does not affect the switching frequency *in vitro* [105].

Curli are thin fimbriae expressed at 26 °C in medium or low osmolarity. Curli expression is dependent on RpoS, the sigma S factor. Transcription of *csgA* (encoding the major structural subunit) can be activated in *rpoS* mutants by inactivation of *hns*, but the temperature and osmolarity control is maintained. Thus, neither of these two proteins is responsible for this control [104]. Olsen et al. [104] concluded that this transcriptional silencing mediated directly or indirectly by H-NS can be relieved by RpoS, or by a non-identified regulatory protein positively controlled by RpoS.

3.1.2. Regulatory role of H-NS in enteroinvasive *E. coli* (EIEC) virulence gene expression

The genes required for EIEC invasiveness are carried on a large virulence plasmid. The ability of invasiveness is temper-

ature dependent, as it is expressed at 37 °C and not at 30 °C. Dagberg et al. [21] have shown that H-NS plays a crucial role in the thermoregulation of virulence-associated genes in EIEC. The VirF protein is a positive regulator of *virG* and *virB* transcription. VirB activates transcription of *ipaBCD* and other genes present elsewhere on the plasmid. IpaB, IpaC and IpaD are secreted proteins essential for invasion. Using *phoA* as a reporter gene, these authors have shown that deletion of *hns* results at 30 and 37 °C in an increase in *virG* transcription but has no effect on *ipaBCD* transcription in the absence of the VirF and VirB activators. By increasing the level of H-NS protein in the cell by cloning *hns* on a low copy vector, the expression of *ipaBCD*, *virG* and *virB* is repressed at 30 °C and to a lower extent at 37 °C, whereas transcription of *virF* is poorly affected. Thus, the higher content of H-NS in the cell causes enhanced thermoregulation. These results suggest that H-NS acts directly on *virG* and *virB* transcription and indirectly on *ipaBCD* transcription via VirB (figure 2). In the absence of H-NS, *virG* expression may become VirF independent. Thus, VirF is a transcriptional regulator which alleviates and/or counteracts the effect of H-NS, as cAMP-CRP, SfaB and SfaC, or RpoS in the case of the *pap*, *sfa*, and *csg* operons, respectively.

3.1.3. Effect of H-NS on haemolysin production

Haemolysin synthesis is repressed at high osmolarities and at low temperatures. Carmona et al. [20] have shown that the mutation in the *hha* gene, encoding a putative histone-like protein, derepresses haemolysin production when cells grow either at low temperature or in a high osmolarity medium. In a *hns* mutant as in the *hha* mutant, *hly* transcription increases about 2-fold compared to the wild-type strain. However, in a *hha-hns* double mutant, haemolysin expression shows a 10-fold increase. Thus, H-NS participates in the modulation of expression

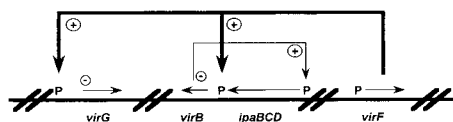


Figure 2. Model for the regulatory interactions of H-NS and EIEC plasmid genes in thermoregulation. H-NS, represented as a dotted circle, acts on the *virG* and *virB* promoters, which are also the target of VirF. The effect seen on the IpaB expression would then be mediated via the VirB expression. Horizontal arrows indicate operons, and vertical arrows indicate site of regulatory action. *p*, promoter; -, negative action; +, positive action (from [21] with permission).

of the *hly* genes. The regulatory mechanism by which this is achieved is not clear but it does not seem to involve modification in the level of DNA supercoiling [93].

3.1.4. Effect of *typA* on H-NS expression

In *E. coli* K12, the inactivation of the *typA* gene encoding the TypA protein (for tyrosine phosphoprotein) alters the expression and the modification of several proteins, such as the disappearance of the universal stress protein UspA, carbon starvation protein Csp15 and the increased synthesis of H-NS [39, 42]. The TypA protein is phosphorylated on tyrosine residues *in vivo* and *in vitro* in the EPEC strain MAR001 but not in K12 *E. coli* strains. The sequence of TypA from the *E. coli* K-12 strain differs from that of an EPEC strain by six amino acid residues and the protein is three amino acids shorter. Freestone et al. [42] concluded that TypA could be a candidate for studying the role of tyrosine phosphorylation in the global regulatory network. BipA of *Salmonella typhimurium* is the homologue of TypA and is implicated in pathogenesis. Another team, Farris et al. [39] reported that BipA/TypA of EPEC is a tyrosine-phosphorylated GTPase that presents a new class of virulence regulators as it controls several processes likely to be important for EPEC infection: the formation of actin-rich pedestals

in host epithelial cells, flagella-mediated cell motility and resistance to the antibacterial effects of a human host defence protein.

3.2. IHF (integration host factor)

IHF is an abundant sequence-specific DNA binding protein that induces a significant bend in the DNA and is involved in a wide variety of processes in *E. coli* including regulation of gene expression. *himA* and *himD* genes encode the subunits of IHF. There is an absolute requirement of IHF for phase variation of type 1 fimbriae which uses site-specific recombination to switch phases. This recombination has an absolute requirement for IHF [37]. IHF also plays a role in the expression of region 1 genes of the type II capsule K5 operon [119]. Binding site consensus sequences were identified in the vicinity of the transcription start site of region 1 of the *kps* cluster. Mutations in *himA* and *himD* led to a 5-fold reduction in the expression of the capsular gene *kpsE* at 37 °C in region 1. This indicates a role of IHF in mediating the expression of region 1 of the capsular gene cluster [119].

3.3. CRP

The global regulator CRP regulates a variety of genes in *E. coli* in response to the level of cAMP which is synthesized by the adenylate cyclase in response to the carbon source. Catabolite repression is due to the inactivation of adenylate cyclase when glucose is transported into the cell. The protein CRP when complexed with cAMP binds to a specific DNA sequence named the CRP-binding site. This sequence occurs at different distances upstream of the transcriptional start site in different operons and leads to the activation of transcription. It is known that cAMP-CRP modulates expression of certain *E. coli* virulence factors such as enterotoxins STa and STb [3, 17] and fimbriae such as Pap, F165₁ and F165₂ pili, colonization factor antigen II, 987P and K99 [22, 36, 46]. The expression of other fim-

briae, such as K88 expressed by ETEC or BFP expressed by EPEC, is, however, not under CRP-cAMP regulation. From that observation, a working model for the temporal and spatial regulation of fimbrial expression in the small intestine by the carbon source was suggested by Edwards and Schifferli [36], where the regulation of fimbrial expression by the carbon source is a major factor in determining the initial site of intestinal binding (see section 2).

3.4. RpoS

rpoS encodes an RNA polymerase sigma factor (sigma S, sigma 38 or KatF). It controls a regulon of 30 or more genes in response to starvation and during transition to the stationary phase. During transition into the stationary phase, the expression of sigma S-dependent genes is activated in a certain temporal order. The sigma S regulon can be divided into subfamilies which include genes regulated by specific stresses and/or additional global regulatory proteins. Subsets of sigma-dependent genes include those genes that are also inducible by anaerobiosis, oxidative or osmotic stress. Products of several of these genes could be considered as virulence factors, including the HpI and HpII hydroxyperoxidases which confer resistances to H₂O₂, as well as CsgA, the main subunit of surface protein curli (see section 8.4) [82]. Maximal acid resistance of *E. coli* MC4100 (and base resistance) is exhibited at the stationary phase and depends on RpoS although RpoS is not an absolute requirement under all growth conditions [122]. For acid resistance, and in part base resistance, the *rpoS* requirement can, however, be overcome by anaerobic growth in moderate acid.

3.5. Lrp

The leucine responsive protein, Lrp, affects the transcription of a large number of genes, increasing the expression of some and decreasing that of others, some of which

are turned on or off by exogenous leucine [92]. It is noteworthy that Lrp is involved in all regulatory mechanisms of fimbriae expression described so far (see section 8). The physiological role of Lrp is unclear. Calvo and Matthews [19] suggest that Lrp positively regulates genes that function during famine and negatively regulates those working during a feast. They propose that pili may be expressed at higher levels during growth in a nutrient-deficient medium where an abundance of pili may help bacterial adherence to epithelial cells, thus helping the maintenance of bacteria in the environment. Under conditions of nutrient excess, bacteria can grow rapidly and should not have any difficulty in maintaining their presence. Thus, abundant synthesis of fimbriae would not be so critical for survival.

3.6. Fur

The exceedingly low availability of iron in mammalian tissues is an environmental signal indicating to the bacteria entry into a host. This signal triggers the co-ordinate expression of bacterial virulence determinants through the Fur protein which plays a general role as a sensor of iron availability in the cell. The apoprotein binds Fe^{2+} as a cofactor, and the cofactor-bound protein binds to various sites, termed iron-boxes. This complex negatively regulates many genes involved in iron uptake as well as toxin genes *hly* and others. The synthesis of the Slt-I toxin, iron-chelating molecules (enterobactin and aerobactin siderophores) and membrane proteins involved in the binding and uptake of iron-siderophore complexes is repressed in low iron conditions [18, 64, 133]. The Fur-iron complex binds to specific DNA sequences, the Fur boxes, located in the operator regions of the iron-regulated operons [18, 25]. Fur does not bind to its DNA target in the absence of iron and genes are thus derepressed in conditions of low iron availability. It is interesting to note that *slt-I* genes are carried by a prophage and are nevertheless controlled

by a chromosomally encoded protein in a way similar to aerobactin (plasmid encoded) and enterobactin (chromosomally encoded). In contrast to the *slt-I* promoter, the *slt-II* and *slt-IIv* promoters do not contain Fur boxes and are not controlled by iron availability [125].

4. FAMILY OF THE ARAC TRANSCRIPTIONAL ACTIVATOR

AraC is the transcriptional regulator of the arabinose operon. Proteins in the family of AraC activator contain helix-turn-helix motifs, which bind specific DNA sequences upstream of genes that are actively transcribed.

The activation of virulence gene expression in EPEC requires an AraC-homologous transcriptional activator protein called PerA/BfpT [45] which is encoded by a virulence plasmid, pMAR2. The genes of the *per* locus are required for transcriptional activation of genes whose products are secreted by the type III secretion system (*eae*, encoding intimin, and *espB*) and they are also involved in activation of plasmidic genes such as the adhesin gene *bfpA* encoding the bundle forming pilus [70, 110, 128]. Insertional inactivation of *perA* led to a reduced expression of Eae which could be due to specific mutations in *perA* or to polar effects on genes downstream of *perA*. Purified PerA/BfpT was shown to bind directly to DNA sequences upstream of *bfpA* and *eae* [128].

Regulatory proteins of some fimbrial operons such as *coo* (CS1), *cfa* (CFA/I), *fap* (987P) and (AAF/1) are designated as Rns-like and share sequence identity with AraC. Rns is required for positive activation of the CS1 fimbrial genes. It was recently shown that Rns is capable of complementing a null mutation in the *S. flexneri virF* gene encoding the homologous counterpart of Rns [108]. The VirF protein cannot, however, complement Rns as an activator of CS1 gene

expression in ETEC. It was concluded that there are differences in the mechanisms by which these related transcription factors regulate gene expression. It is not known whether the Rns class of regulatory proteins binds DNA or whether there are additional factors in the regulatory network [78].

5. SENSOR REGULATORY PROCESSES

Environmental signals control virulence gene expression in bacteria. A developing research field is understanding the ways in which bacteria sense these signals and transduce them into the cell to regulate gene expression. Many signal response systems in bacteria operate by complex pathways involving two-component regulatory systems. These systems consist of: 1) the sensor protein which spans the cytoplasmic membrane and monitors some environmental parameters; and 2) the regulator protein that mediates an adaptive response, usually by a change in gene expression. When the sensor component receives an external signal, it undergoes autophosphorylation and transfers the phosphate residue to the regulator protein which in turn activates or represses gene transcription. Several two-component systems controlling virulence gene expression have been well characterized in different bacteria [40]. In *E. coli*, the expression of some group IK antigens as well as colonic acid is regulated by the RcsABC (regulator of capsule synthesis) system. A temperature below 25 °C, the presence of a high phosphate concentration and osmotic induction increase the synthesis of these antigens [121]. The integral inner membrane RcsC sensor and the cytoplasmic RcsB effector show homologies to the family of the two-component histidine kinase signalling systems [48]. The mechanisms whereby extracytoplasmic signals are sensed and transduced by the RcsC membrane sensor are not unknown. Presumably, after receipt of a stimulatory signal, RcsC

first autophosphorylates at a reactive histidine residue, then subsequently transfers the phosphate to a conserved aspartate on RcsB. This form of RcsB may form a more stable complex with RcsA and promotes *cps* transcription perhaps aided by RcsF [48, 50, 69]. RcsA is an additional positive regulator and is subject to degradation by the Lon protease [68].

Effective mechanisms for the induction or repression of virulence gene expression involve the sensing of 'signature' molecules produced by host tissues. In this way, the *E. coli* regulons SoxRS and OxyR are designed to deal with the cytotoxic products produced during the oxygen-dependent respiratory burst, with a large number of proteins being induced in response to hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻) or nitric oxide (NO) produced by neutrophils and macrophages [82, 99]. The SoxRS regulon controls the expression of approximately ten genes. SoxR is an iron sulphur protein that senses exposure to superoxide and nitric oxide, and then activates the transcription of the *soxS* gene. SoxR and SoxS are DNA-binding proteins, SoxR being a member of the MerR-like family (MerR is involved in the response to Hg²⁺) and SoxS being a member of the AraC-family. Transcription of *soxS* is initiated in a manner dependent on the *rpoS* gene in response to entering stationary phase growth [98]. The SoxS protein then activates the transcription of a variety of genes including *sodA*, encoding the Mn-dependent superoxide dismutase, *nfo*, encoding the DNA repair enzyme endonuclease IV, and *micF*, which post-transcriptionally decreases the production of OmpF [82].

OxyR, a member of the LysR family of autoregulators, is also involved in resistance to oxygen-related compounds. The OxyR protein activates the transcription of approximately nine genes in *E. coli* in response to H₂O₂. These include *katG*, encoding HP1 catalase, and *ahpFC*, encoding NADPH-dependent alkyl hydroxperoxidase, *gorA*, encoding a glutathione reductase and *dps*

encoding a protein that non-specifically binds to DNA to protect cells from H_2O_2 toxicity. OxyR functions both as a sensor and a transducer and contains a critical redox-sensitive Cys residue that is oxidized by hydrogen peroxide [117, 139]. The level of *oxyR* mRNA or OxyR protein does not change significantly following exposure of the cells to H_2O_2 . Hence, it was suggested that post-translational regulation by an H_2O_2 -generated signal activates the pre-existing OxyR protein [80].

P pili-mediated attachment may also be an important part of sensor regulatory processes involved in uropathogenic *E. coli* during urinary tract infection. Zhang and Normark [138] have shown that *airS* transcription is specifically activated by P pili attachment. The AirS protein is a sensor-regulator protein essential for the iron-starvation response of uropathogenic *E. coli* that activates the synthesis of the siderophore iron acquisition system. Furthermore, the two-component signal transduction system, CpxA-CpxR, has been suggested to play a role in the expression of virulence factors of *E. coli* via P pili attachment. Hultgren [61] hypothesized that the adhesion of P pili to epithelial cells avoids polymerization of the pilin fibre and thus leads to aggregation of misfolded pilus subunits in the periplasm. Protein aggregation in the periplasm is sensed by the CpxA-CpxR system, which activates *htrA* encoding DegP (a periplasmic protease degrading misfolded proteins) [23, 107], and could be involved in controlling expression of virulence factors such as haemolysin or CNF (cytotoxic necrotizing factor). Thus, the interaction between the pathogen and its host receptors mediated by adhesins may be a means for bacteria to sample and consequently elicit the appropriate response upon their arrival at a potential colonization site. Thereby, bacterial attachment to mucosal surfaces via P pili indicates that bacteria have reached an ecological niche where expression of virulence factors (siderophores, toxins) are necessary for survival.

6. tRNA REGULATION OF VIRULENCE GENES

Many virulence genes are located on 'pathogenicity islands' (Pais), large chromosomal regions that are often associated with particular tRNA genes [52]. It has been shown that the Pais modulate virulence gene expression through the action of these tRNA [112]. In *Shigella*, it has also been demonstrated that expression of tRNA₁^{tyr} partially complemented the *virR* mutation. *virR* is an analogue of *hns* and is required for co-ordinating temperature-regulated virulence gene expression of *Shigella* [57].

The uropathogenic *E. coli* strain 536 (06 :K15 :H31) carries two pathogenicity islands, one Pai comprising the gene cluster of haemolysin and the other Pai comprising the gene clusters of haemolysin and P-related fimbriae, both Pais being flanked by tRNA genes, *leuX* and *selC*, respectively. In the uropathogenic strain 536, spontaneous deletions resulting in the truncation of *leuX* or the specific deletion of the tRNA₅^{leu} gene resulted in the lack of expression of type 1 fimbriae and other virulence factors such as haemolysin, aerobactin production, serum resistance phenotype and motility, while trans-complementation of tRNA loci leads to a restoration of these properties [91, 127]. tRNA₅^{leu} is specific for the minor leucine codon UUG. It has been shown that tRNA₅^{leu} is required for efficient translation of FimB whose gene contains five TTG codons recognized by tRNA₅^{leu} that in turn leads to type 1 fimbrial expression [113].

The selenocysteine-specific tRNA (*selC*) directly influences the ability of the *E. coli* strain 536 to grow under anaerobic conditions because selenocysteine is part of the formate dehydrogenase (FDH) enzyme which is involved in mixed acid fermentation. The ability to grow under anaerobic conditions via mixed acid fermentation may be important for the colonization of *E. coli* in the kidney because oxygen-limiting conditions are found in deeper regions of the kidney [112].

7. BLACK HOLES AND VIRULENCE GENE EXPRESSION

Not only deletion of genes such as *leuX* can affect the expression of virulence, but addition of some genes can also alter the virulence of bacteria. It was observed that *Shigella* and enteroinvasive *E. coli* displayed deletions in the *cadA* region present in most *E. coli* strains including *E. coli* K12 [84]. These deletions have been termed black holes and they enhance the expression of virulence genes. Indeed the introduction of the *cadA* gene encoding lysine decarboxylase attenuates the virulence of *Shigella flexneri* and the enterotoxin activity was inhibited by cadaverine, a product of the reaction catalysed by lysine decarboxylase.

8. REGULATION OF FIMBRIAL EXPRESSION

Synthesis of fimbriae is under complex regulatory controls. First, assembly of fimbriae depends on the appropriate levels relative to one another of major and minor fimbrial subunits, the chaperon, outer membrane pore and regulatory proteins. The major fimbrial subunit must be expressed at higher levels than other accessory proteins. Fimbrial determinants are organized in operons and differential expression of genes is achieved by post-transcriptional mechanisms. In the *pap* operon, encoding P fimbriae, the two first cistrons, *papA* and *papB*, are co-transcribed to mRNA from the *papB* promoter. Differential expression of these two genes results from RNase E-dependent endonucleolytic cleavage of the mRNA in the intercistronic *papB-papA* region. This cleavage is followed by rapid decay of the upstream *papB*-encoding region and accumulation of the stable *papA*-encoding mRNA, leading to high level expression of the major subunit, PapA, relative to that of the regulatory protein PapB [94]. Differential mRNA stability and mRNA processing are also involved in the control of the *daa*

operon (encoding for the F1845 fimbriae) [12]. Transcriptional organization of the *daa* operon is quite different from that of *pap* since the major fimbrial subunit gene *daaE* is located at the 3' end of the operon. A large transcript encoding accessory proteins in addition to the major subunit is submitted to endoribonucleolytic cleavage to generate a stable *daaE* mRNA. This cleavage requires neither RNase III nor RNase E. Second, expression of fimbriae is controlled by environmental signals through alterations in transcript levels. The control of fimbriae expression is of crucial importance for the survival of bacteria in vivo [35]. It allows fimbriae to be expressed only at the adequate site in the host and to evade the host's immune response. All fimbrial operons examined so far encode specific regulatory proteins that either activate or repress transcription. In addition, global regulators such as Lrp, CRP, H-NS, IHF, RpoS, play roles in controlling the expression of most fimbrial operons. Four different regulatory mechanisms controlling the expression of fimbriae have been described. Operons that share common regulatory properties are classified in *table 1*.

8.1. The *pap* regulatory family

Regulation of the *pap* expression is the most extensively studied. Expression of P fimbriae is subject to phase variation, i.e. fimbrial expression switches between ON (fimbriae-positive cells) and OFF (fimbriae-negative cells) states. The switch frequency is controlled by environmental factors such as temperature or carbon source. Phase variation of P fimbriae depends on the methylation status of two GATC sites located in the intercistronic region between the regulatory genes *papI* and *papB* which are transcribed divergently (*figure 3*). The two GATC sites, GATC-I and GATC-II, are located within the Lrp-binding sites. Schematically, when Lrp binds the non-methylated GATC-II site, transcription from

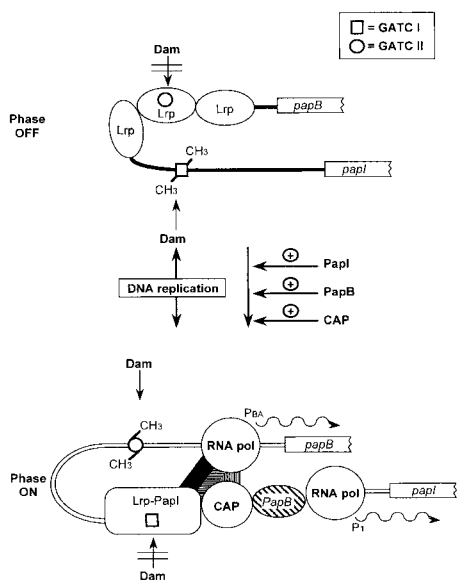


Figure 3. Pap phase variation model. Transcriptionally active (phase ON) and inactive (phase OFF) states of *pap* are shown. The transition between these transcription states is thought to require DNA replication [14, 16]. The GATC-I and GATC-II sites are represented by a square and a circle, respectively. Methylation of these GATC sites in the ON and OFF states is indicated by '-CH₃'. Proteins involved in *pap* transcriptional regulation are shown, as well as the *papI* and *papB* genes. Dam, deoxyadenosine methylase; RNA pol, RNA polymerase holoenzyme; Lrp-PapI, complex between the Lrp and PapI regulatory proteins. Hatched areas indicate putative interactions occurring between regulatory proteins and RNA polymerase. The *papBA* and *papI* transcription start sites are shown by wavy lines (from [78] with permission).

the pB promoter is locked and cells are in the OFF state. Under conditions of high cAMP levels, PapI is expressed and binds to Lrp, resulting in a shift in Lrp binding from the GATC-II site to the GATC-I site. Binding of Lrp-PapI to GATC-I activates transcription from the pB promoter and switches the cells to the ON state. The switch between the

OFF and ON states requires DNA replication to alter the methylation status of GATC-I, because binding of Lrp-PapI to this site is inhibited by full methylation. PapB activates *papI* transcription and represses *papBA* transcription (figure 3) [15, 66, 67, 95, 96, 130, 132].

Several other fimbrial operons are controlled by analogous mechanisms with slight variations. *sfa* (*S. fimbriae*), *daa* (F1845), *clp* (31A), *fae* (K88), *foo* (F165₁) operons encode for proteins with significant homologies to PapI and PapB [22, 60, 83, 87]. Their expression is controlled by Lrp and Dam methylation. *daa*, *sfa*, *foo* and *clp* operons are under phase variation control but *fae* is not. Lrp is required for the OFF to ON switch of the phase variation-controlled operons. *clp* and *foo* are the only operons described so far that belong to this regulatory family in which expression is modulated by the level of leucine and alanine in the growth medium. It has been shown that these amino acids totally inhibit the OFF to ON switch of *clp* but the precise molecular mechanism is not yet known [83]. Lrp and the specific regulators are not only involved in phase variation control but also in the regulatory mechanisms controlling the level of transcription in phase ON cells or in cells not subjected to phase variation. Martin [83] has shown that Lrp and ClpB repress *clp* transcription in phase ON cells and that this effect requires Dam methylation. Lrp also acts as a negative regulator of the *fae* operon which is not under phase variation control [60].

Several *E. coli* strains possess determinants for the synthesis of two or more fimbriae. Cross regulation of two unlinked fimbrial operons belonging to the same regulatory family has been demonstrated. Regulatory *prfI* and *prfB* genes of the *pef* operon encoding for P-related fimbriae are able to complement mutations in *sfaC* and *sfaB* regulatory genes in a uropathogenic *E. coli* isolate. PrfI and PrfB share 87 and 76 % homology with SfaC and SfaB, respectively. Moreover, mutations in *prfI* and *prfB* lead to significant reductions in *sfa* transcription [88].

Table I. Genetic and regulatory classification of *E. coli* fimbrial operons found in strains isolated from animals modified from Low et al. [78].

Fimbrial operon	Fimbrial type	Operon-encoded regulatory protein	Global regulator	Population phenotype	Environmental responses	References
<i>pap</i>	P	PapI ^a , PapB ^b	Lrp, CRP, Dam, H-NS	phase variable	carbon source, temperature, solid versus liquid growth medium	[14–16, 95, 96, 132]
<i>foo</i>	F165.1	FooI ^a , FooB ^b	Lrp, CRP	phase variable	carbon source, leucine, temperature	[22], unpublished results
<i>daa</i>	F1845	DaaF ^a , DaaA ^b	Lrp, Dam, IHF	phase variable	temperature, carbon source	[130]
<i>clp</i>	CS31A	ClpI ^{a?} , ClpB ^b	Lrp, Dam	phase variable	solid versus liquid growth medium, temperature, leucine, alanine	[44, 83]
<i>fae</i>	K88	FaeA ^a , FaeB ^b	Lrp	uniform	temperature, growth phase	[59, 60]
<i>sfa</i>	S	SfaC ^a , SfaB ^b	Lrp, Dam, H-NS	phase variable	carbon source, temperature, O ₂ , growth rate, solid versus liquid growth medium, osmolarity	[86, 87, 118, 130]
<i>fas</i>	987P	FasH	CRP, H-NS	phase variable	carbon source, temperature, pH, nitrogen source, growth rate, O ₂	[36, 72, 131]
<i>csg</i>	Curli	Crl	RpoS, H-NS	ND	temperature, osmolarity	[5, 53, 103, 104]
<i>fim</i>	Type 1	FimB, FimE	Lrp, H-NS, IHF, RpoS	phase variable	temperature, leucine, alanine, liquid versus solid growth medium	[13, 27, 32, 43, 114]
<i>fan</i>	K99	FanA ^b , FanB ^b	Lrp, CRP	uniform	carbon source, temperature, pH, growth rate	[15, 65, 115]

A same superscript designates homologous regulatory proteins. ND, non-determined.

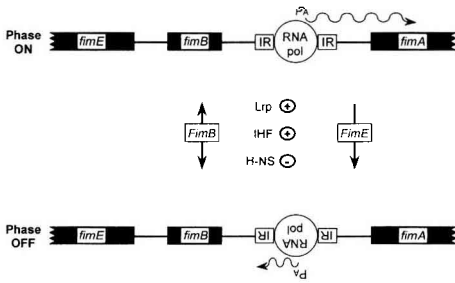


Figure 4. Type I fimbrial phase variation model. Transcriptionally active (phase ON) and inactive (phase OFF) states of *fim* are shown. The inverted repeats region (IR) consists of the 9-bp sequence 'TTGGGGCCA', which flanks a 314-bp DNA fragment containing the transcription start site for *fimA* designated as PA. The *fimA* gene codes for the major fimbrial subunit, whereas *fimB* and *fimE* are regulatory genes required for inversion-dependent phase variation. The effects of Lrp, IHF and histone-like protein H-NS on *fim* inversion are shown (from [78] with permission).

8.2. Regulation of type I fimbriae expression

Phase variation controls the expression of type I fimbriae (*fim* operon) by site-specific recombination. Recombination leads to the inversion of a 314-bp element. According to the orientation of the promoter, fimbriae are either produced or not produced. The inversion process is carried out by two integrase-like proteins, FimB and FimE. FimB is involved in ON to OFF and OFF to ON inversion, FimE only in ON to OFF inversion [2, 73]. In addition, four global regulators modulate the *fim* switch: Lrp, IHF, H-NS and RpoS (figure 4).

Lrp stimulates the *fim* inversion in a Dam-independent manner. Lrp slightly increases the transcription of *fimB*, decreases *fimE* transcription by 2-fold [13], and binds with high affinity to the switch element in vitro [43]. Leucine potentiates the effect of

Lrp on the switch. The model of Roesch and Blomfield [114] assumes that leucine promotes the selective dissociation of Lrp from one DNA binding site within a multimeric nucleoprotein complex, resulting in a structure that is more favourable for recombination than that produced by the Lrp bound to all binding sites.

The integration host factor (IHF) is necessary for the *fim* inversion. IHF could play a direct role in site-specific recombination or it could modulate transcription of *fimB* and/or *fimE* [31, 37].

The histone-like protein H-NS has a negative influence on the rate of recombination. In a *hms* mutant, transcription of both *fimB* and *fimE* is enhanced [101]. Donato et al. [27] have shown that H-NS interacts directly with the *fimB* promoter sequences. This results in a promoter-specific repression.

The alternative sigma factor RpoS exerts a negative control on *fimA* (encoding the major fimbrial subunit) and *fimB* promoters by an unknown mechanism and influences DNA inversion negatively in broth cultures [32].

8.3. Regulation of K99 expression

The regulation of K99 expression is less well understood. The K99 determinant encodes eight genes organized in three transcriptional units. Region 1 encodes *fanA–D*, region 2 *fanE–F*, region 3 *fanG–H* [65]. The *fanC* gene product is the major fimbrial subunit, whereas products of *fanA* and *fanB* are related to each other and to PapB. None of the *fan* genes is related to *papI* [115]. K99 expression is not controlled by phase variation but is repressed by leucine and alanine. The global regulators CRP and Lrp are both required for K99 expression [15, 65]. Lo-Tseng et al. [77] demonstrated that Lrp affects only the transcription of region 1 genes, whereas Inoue et al. have shown that CRP is required for expression of region 1

and 2 genes [15, 65]. Differential methylation due to Lrp is, however, not involved in the regulation of K99 expression, as it is for the fimbriae of the P regulatory family. The mechanism by which leucine and alanine affect K99 synthesis remains unknown. The role of FanA and FanB remains obscure. Frameshift mutations in FanA or FanB reduced the level of K99 production 8- and 16-fold, respectively, but did not have much effect on transcription from the *fanA* promoter.

8.4. Regulation of curli expression

A new type of fimbriae has been described, whose expression is maximal in conditions which are characteristic of an extraintestinal environment, i.e. low temperature (26 °C) and low osmolarity. These fimbriae, referred to as curli [102], are synthesized by pathogenic and non-pathogenic *E. coli* as well as by other Gram-negative bacteria [28]. They bind fibronectin, plasminogen, different sera and tissue proteins and the dye Congo red. Curli formation involves a novel and a so far unique assembly mechanism not yet completely understood.

Two divergently transcribed operons are necessary for curli formation, *csgBA* and *csgDEFG* [53]. CsgA, the fibre subunit, is first secreted in the extracellular milieu as a soluble protein and then polymerized at the cell surface in a CsgB-dependent manner [54]. CsgG is an outer membrane-located lipoprotein required for stable maintenance of CsgA and CsgB [76]. CsgD is a transcriptional activator of the *csgBA* promoter belonging to the LuxR family [53]. Regulation of curli expression is complex and involves at least two global regulators, H-NS and RpoS. So far, no report presents evidence for a role of Lrp in this regulation. Thus, Lrp seems to be involved in regulating expression of fimbriae synthesized in conditions that mimic the in vivo environment. Curli are produced in the stationary phase.

Transcription of *csgBA* and *csgDEFG* is dependent upon RpoS in wild-type cells, but not in a *hns* background [53, 104]. The requirement of RpoS for transcription of *csgBA* may be indirect via CsgD with CsgD being necessary for *csgBA* transcription. Temperature and low osmolarity control of fibronectin binding as well as growth phase control of *csgBA* transcription are maintained in a *rpoS/hns* double mutant, indicating that neither H-NS nor RpoS is responsible for these regulatory responses [5, 53]. Considering that CsgD is required for *csgBA* transcription and is produced in a *hns/rpoS* double mutant, Hammar et al. [53] hypothesized that CsgD may have the capability to respond to starvation signals and/or high cell density by activating *csgBA* transcription. This is a seductive hypothesis since CsgD belongs to the LuxR family of transcriptional activators involved in quorum sensing regulation (see in section 11).

9. REGULATION OF HAEMOLYSIN EXPRESSION

E. coli haemolysin (Hly) is a well-characterized member of the RTX family of cytotoxins associated with urinary tract infections and other extra-intestinal *E. coli* infections [134, 135]. Synthesis of Hly is directed from an operon consisting of four contiguous genes, *hlyCABD*. The *hlyA* gene encodes the component of haemolysin, which undergoes HlyC-dependent acylation. Sec-independent haemolysin secretion requires HlyB and HlyD. Two transcripts initiating at the same promoter located upstream of *hlyC* are synthesized: a major *hlyCA* mRNA and a minor *hlyCABD* mRNA. The higher stability of the major transcript accounts in part for the differential expression of HlyC and HlyA relative to HlyB and HlyD. Hly expression is regulated by several mechanisms involved in mRNA stability, DNA supercoiling, transcription elongation and activity of the product.

9.1. DNA supercoiling

Hly expression is modulated by environmental factors such as osmolarity, temperature and anaerobiosis [89]. Low temperature and high osmolarity repress haemolysin expression. The Hha protein is representative of a new class of modulators of gene expression in enterobacteria [24]. Carmona et al. have shown that Hha influences DNA topology and suggest that it is a histone-like protein [20]. They have established a relationship between the *hha* mutation and an increase in haemolysin synthesis through changes in DNA topology. The Hha protein participates in the temperature- and osmolarity-dependent regulation of Hly expression in a manner that strikingly resembles what has been shown for H-NS with other virulence determinants. The *hha* mutation significantly increases expression of *hly* genes in high osmolarity media and at low temperatures [90]. The Hha protein is highly similar to the YmoA protein from *Yersinia enterocolitica*. The *hha* gene can complement the *ymoA* mutation and vice versa [9, 85]. The complementation appears to be dependent on gene dosage [9].

9.2. Transcription elongation

hlyCABD transcription elongation is modulated by the RfaH protein which controls a regulon governing the synthesis, export and assembly of cell surface and extracellular components that influence DNA transfer and virulence: RfaH is a positive regulator of *rfa* (encoding the LPS core), *tra* (encoding the F pili) and *kps* (encoding the production of type 2 capsule) gene expression [8]. RfaH abolishes transcriptional polarity within a transcript, increasing transcription of distal genes in operons. The non-encoding regions of all RfaH-affected operons contain all or a portion of a conserved 39-bp sequence named JUMP-Start. The *ops* element is the second half of a direct repeat in the JUMP-Start

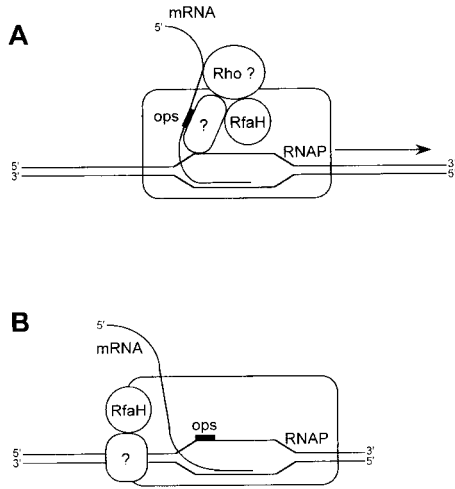


Figure 5. Speculative models of the mode of action of RfaH and the *ops* element.

A. Recognition of the *ops* element as an RNA sequence. The *ops* element (black box) is recognized by an unknown factor(s) (labelled?), which binds to RfaH, the elongating RNA polymerase (RNAP; large box) and possibly the termination factor Rho to give a termination-resistant complex that transcribes 3' of the *ops* element (arrow). **B.** Recognition of the *ops* element as a DNA sequence. The RNAP pauses on *ops* element, and the paused complex and the *ops* element is recognized by unknown factors (?) and RfaH. These factors then modify the polymerase complex. This allows the release of the termination-resistant complex from the pause site to continue transcription. The *ops* element is drawn here in the 'transcription bubble' but might be present 5' of the bubble and could be recognized by the unknown factors and RfaH as either double- or single-stranded DNA (from [8] with permission).

sequence. Bailey et al. [8] propose that the *ops* element recruits the RfaH protein to the transcription complex. Then the RfaH-influenced elongating RNA polymerase complex resists transcription termination signals downstream of the *ops* element (figure 5). This model is supported by the significant amino acid similarities between RfaH and NusG, an essential transcription elon-

gation factor required for effective anti-termination by the N protein of bacteriophage lambda. In the *hly* operon, transcriptional polarity is due to a Rho-independent terminator between *hlyA* and *hlyB* genes. Cooperation between RfaH and the cis acting *ops* element enables the RNA polymerase complex to proceed through the *hlyA-hlyB* intergenic terminator [7].

9.3. Stabilization of the active conformation of the toxin

LPS may play a role in protecting the secreted HlyA protein from degradation. Removing the core sugars from LPS affects the kinetics and stability of secreted haemolytic activity. The model proposed by Bauer et al. [11] suggests that Hly exists as a complex including LPS. These molecules combine to form an active toxin whose stability is aided by the LPS inner core. When the inner core is incomplete, LPS and Hly form large, inactive aggregates, and render Hly more susceptible to decay.

10. REGULATION OF CAPSULE SYNTHESIS

Group I K antigens are characterized by a high molecular mass (over 100 kDa) and a low charge density. The coexpression of the group I K antigens and LPS is restricted to a few LPS serotypes (primarily 08, 09 and 020) [111]. Expression of some group I K antigens as well as colonic acid is regulated by the RcsABC (regulator of capsule synthesis) system (see section 5). A membrane-anchored DjIA protein, a member of the DnaJ 'J-domain' family, acts in concert with RcsB/C two-component system to increase induction of the *cps* (capsular polysaccharide) operon [69]. Moreover, the *cps* operon activation by DjIA is dependent upon DnaK (Hsp70) and GrpE, which are parts of a chaperon machine.

Group II K antigens have a lower molecular mass and a higher charge density than group I K antigens, are coexpressed with a variety of LPS and expressed at 37 °C (at the physiological temperature). K1 and K5 *kps* clusters have been determined. The synthetic, regulatory and export components for capsule expression are encoded in three functionally distinct gene blocks [111]. The *kps* cluster of K1 is functionally divided into three regions. These regions are organized as two convergently transcribed operons inserted into a monocistronic tRNA gene *pheV*. The central region, region 2, contains a biosynthetic cassette that is flanked on either side by regions 1 and 3 whose genes function in more general aspects of capsule biosynthesis. Mutations in either region 1 or region 3 tend to cause accumulation of intracellular polysaccharides within the cell. This suggests that these regions are important for the transport of polysaccharides to the cell surface. The six genes of the region 1 operon are transcribed in the same direction as *pheV*.

Region 1 of the K5 capsular gene cluster in which *kpsFEDUCS* genes were identified is transcribed as a single transcriptional unit that is processed to yield a smaller transcript specific for the *kpsS* gene which is at the 3' end of the transcript [119]. Processing of mRNA appears to be implicated in a differential expression of *kps* genes. Mutations in the *himA* and *himD* genes which encode the subunits of IHF led to a reduction in the expression of KpsE at 37 °C [119]. Region 1 is submitted to a thermoregulation with no transcription at 18 °C. Expression of the region 1 operon is thermoregulated by transcriptional control of its first gene, *kpsF* [119]. It appears that regulation of region 1 expression in response to temperature is mediated neither by the *hns*, *rimJ* nor *hha* gene products, all of which have been implicated in temperature-dependent regulation gene expression in other systems [119].

A mutation in the *rfaH* gene abolishes the K5 capsule expression at 37 °C [123]. Expression of region 1 is not mediated by

RfaH, which is required for expression of region 3 at 37 °C and the expression of *rfa* genes [119, 123]. Stevens et al. [124], proposed a model where RfaH regulates expression of the *E. coli* group II capsule gene clusters by allowing readthrough transcription to proceed from region 3 into region 2. The non-encoding region upstream of the clusters involved in the production of various polysaccharide antigens contains a 39-bp sequence (a sequence upstream of *kspM* of region 3 of the *kps* cluster, outer core *rfa*, O-antigens *rfb* of LPS). This was referred to as the JUMP-Start sequence and it has been postulated to be involved in the transcriptional regulation of bacterial gene clusters encoding surface proteins. The JUMP-Start sequence could cause RfaH-dependent antitermination at other Rho-dependent terminators suggesting that the JUMP-Start sequence may function, in a manner analogous to a lambda *nut* site, in the regulation of bacterial gene clusters encoding surface polysaccharides (see section 9.2) (figure 5).

11. PERSPECTIVES

The regulation of the expression of virulence genes has mainly been studied *in vitro*. Nowadays, with the advent of new genetic methods, the identification of the regulation mechanisms that are induced *in vivo* as well as the determination of their activities on virulence factors in a given location within the host are now ongoing. New methodologies for the study of *in vivo* gene expression allow the isolation of bacterial genes expressed during infection [55, 106]. Using genetic reporter fusions, genetic expression at the cellular level within infected animals could be examined [40]. Through the use of reporter molecules such as the green fluorescent protein and others under development, it will be possible to explore microbe–host interaction in real time in the living host [40].

Effective mechanisms for the induction of virulence genes could involve the sensing

of ‘signature’ molecules produced by host tissues [82]. Probing the host environment with tools that define the genes expressed by bacteria during infection will provide a better definition of the microbial–host interaction at both ends of the spectrum. At a higher level, one needs to understand how signals are detected and interpreted to influence gene expression and what are the effector signals produced by the host tissues. More specifically, since several type III secretion systems of attaching and effacing *E. coli* are activated by the contact of the bacteria with the surface of eukaryotic cells, the questions raised are: what is the nature of eukaryotic cell surface signals that activate the type III secretion systems, and what are the regulatory proteins involved in the regulation of these secretion systems [58]?

Another aspect of *E. coli* virulence factor expression that is under scrutiny is the role of the population density detection in *E. coli* virulence. In pathogenic bacteria, such as *Pseudomonas*, quorum sensing plays a defined role in virulence. Quorum sensing is a phenomenon by which bacteria sense and respond to their own population density by releasing and sensing pheromones. In Gram-negative bacteria, quorum sensing is often performed by the LuxR family of transcriptional regulators, which affect phenotypes as diverse as conjugation, bioluminescence and expression of virulence genes. In Gram-negative bacteria the most common form of quorum sensing is mediated by the production and subsequent perception of autoinducers, the acylated homoserine lactones (acyl HSLs). By sensing the density of a secreted autoinducer, bacteria can sense if there is a quorum of their population sufficiently present to initiate the appropriate biochemical reaction. Homologues of LuxR, such as SdiA (suppressor of cell division inhibition) and CsgD have been identified in *E. coli* [53, 120]. SdiA-mediated autoinduction with RpoS regulates *fisQA* whose products are involved in cell division [120]. Recently, by using the *Vibrio harveyi* mutant that responds exclusively

to an uncharacterized signal molecule AI-2, Surette and Bassler [126] demonstrated that cell-free culture fluids of *E. coli* and *S. typhimurium* can contain high levels of AI-2-like factors. The role of this putative autoinducer and SdiA in quorum sensing for pathogenic *E. coli* and the regulation of the expression of virulence factors remains to be determined.

Given that most virulence genes appear to be regulated, regulatory systems are obvious targets for the development of new therapeutic drugs. The identification of molecular targets including regulators associated with adaptation/survival and pathogenesis offers the prospect of expanding the horizons of anti-infective therapy beyond the confines of agents that are merely bactericidal or bacteriostatic [74]. It could increase the level of therapy beyond that currently possible with antibiotics alone. Thus, a better understanding of the regulation mechanisms that govern the expression of virulence factors not only *in vitro* but also temporally and spatially within the host will allow the development of new therapeutic approaches.

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