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Regulatory integration of horizontally-transferred genes in bacteria

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TABLE OF CONTENTS

1. ABSTRACT

Horizontal transfer of genetic material is a fact of microbial life and bacteria can obtain new DNA sequences through the processes of conjugation, transduction and transformation. This offers the bacterium the possibility of evolving rapidly by importing new genes that code for new traits that may assist in environmental adaptation. Research in this area has focused in particular on the role of horizontal transfer in the dissemination through bacterial populations of genes for resistance to antimicrobial agents, including antibiotics. It is becoming clear that many other phenotypic characteristics have been acquired through horizontal routes and that these include traits contributing to pathogenesis and symbiosis. An important corollary to the acquisition of new genes is the problem of how best to integrate them in the existing gene regulatory circuits of the recipient so that fitness is not compromised initially and can be enhanced in the future through optimal expression of the new genes.

2. INTRODUCTION

A pioneering step in the discovery of horizontal gene transfer was made when Griffith described the lateral transfer of virulence traits between pneumococci (1). Subsequently it was shown that this process of 'transformation' involved lateral movement of genetic material between bacterial strains (2). There are at least three mechanisms by which modern bacteria acquire DNA horizontally (3, 4) (Figure 1). The first is conjugation, a process in which a self-replicating extrachromosomal element (usually a plasmid) organizes its own transfer from one cell to another through a mating bridge consisting of a hollow contractible proteinaceous tube whose subunits are encoded by the mobile genetic element. In most cases, the recipient is also a bacterium. However, it should be pointed out that some conjugation processes involve transfer of bacterial DNA to eukaryotic cells (5, 6). The second lateral gene transfer mechanism is transduction, which involves genetic transfer via a bacterial virus or bacteriophage (7).

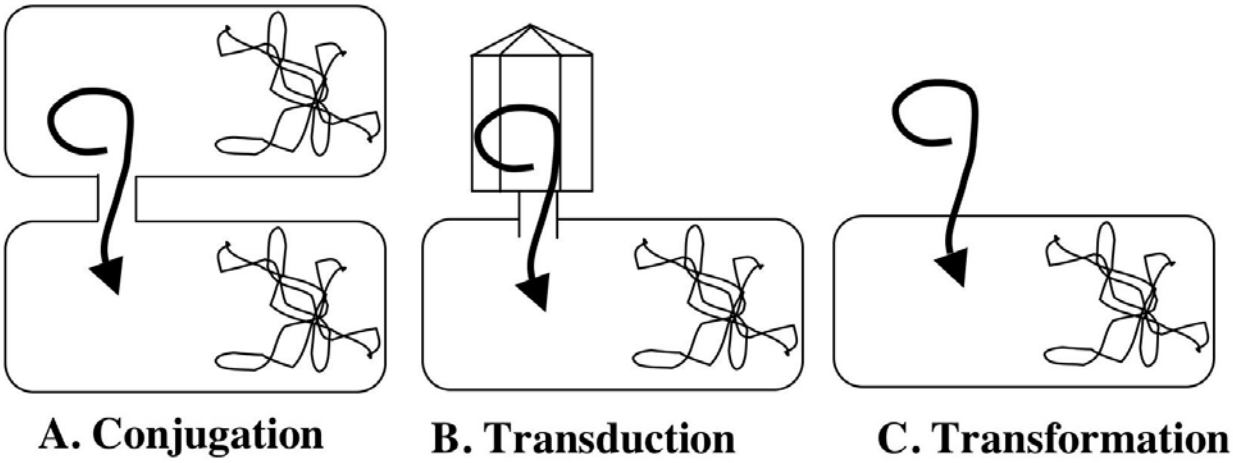

Figure 1. A summary of the principle mechanisms of horizontal gene transfer used by bacteria. Conjugation (A) involves the self-transmission of a plasmid from one bacterial cell to another through a contractible mating pilus. In transduction (B), a bacteriophage acts as the vehicle for DNA transmission. The genetic material stored in the phage head is injected into the bacterium through a contractible tail structure in the virus. The process of transformation (C) involves no vehicle. Here the genetic material is taken up by the bacterium directly from the external environment. Not to scale.

Here DNA is packaged within the phage head and injected into a new bacterial host following receptor-mediated absorption of the virus onto the outer surface of the recipient cell. The third process is transformation, in which naked DNA is taken up by the bacterium directly from the external environment (3, 4). Some bacteria are proficient for uptake (i.e. are transformable) all of the time while others require particular circumstances to make them susceptible.

The fate of the newly-acquired DNA is partly determined by its relationship to the DNA of the new host. Restriction systems exist to protect bacteria from foreign DNA and if the new genetic material lacks the pattern of chemical modification (usually methylation) that is a characteristic of its own DNA, the restriction enzymes will cut it up. However, this protection has been described as both limited and short lived (8, 9). Restriction systems require that the invading DNA is double-stranded; single stranded DNA will not be cleaved (10). Some plasmids can defeat restriction systems in their new hosts using a function known as ARD (alleviation of restriction of DNA) (11). Many phage lack recognition sites for restriction enzymes or have evolved ways of masking them; they may also alter the activities of restriction systems to render them harmless to the virus (8, 12). For these and other reasons, some foreign DNA will survive and any genes associated with it may become expressed. However, if these genes are to be inherited by future generations of the bacterium, they must be replicated. As autonomously-replicating genetic entities, plasmids are at an advantage here. Some phage can replicate autonomously too, and have plasmid-like characteristics. The phages P1 and P7 represent examples of viruses of this type and they have been studied intensively (13, 14, 15). Other phage integrate into the chromosome and replicate as a part of the bacterial genome. Bacteriophage lambda is a familiar example of a temperate phage that displays this type of behavior. Here entry into the chromosome involves recombination between specific sites on the bacterial chromosome and on the viral genome (16, 17, 18). The integrated form of the virus is called a prophage and microbial genome sequencing projects have revealed that prophage are common components of many bacterial genomes (19-22). In many cases the phage discovered by genome sequencing are vestigial, having lost the potential to form infectious virus particles. Nevertheless, many contain genes that contribute to the lifestyles of their modern bacterial hosts. In some cases these include genes with roles in bacterial virulence (19, 23, 24).

DNA that arrives in the cytoplasm following transformation is likely to be degraded quickly if it does not become part of a replicon (4). Some measure of DNA sequence homology with part of the genetic complement of the new host is an aid to recombination. However, even an unrelated sequence may be integrated by illegitimate recombination, albeit at a low frequency (25, 26).

Plasmids and bacteriophage are classed as mobile genetic elements, a term that is also applied to genetic entities such as insertion sequences, transposons and integrons. Interestingly, the restriction-modification systems that offer protection from laterally transferred DNA sequences have themselves been classified as selfish mobile genetic elements (27, 28) that have been distributed among bacterial populations by horizontal gene transfer (29). The boundaries between different types of mobile element often appear to be arbitrary and reflect the history of their discovery and characterization. For this reason, individual mobile elements may possess features normally associated with elements from other groups. There are many well-known examples of this in the literature. For example, some phage, such as the P1 and P7 viruses referred to above, replicate as low-copy number

plasmid/prophage (30); some transposons are conjugative and so share this property with self-transmissible plasmids (31); others, such as transposon Tn*7*, have a site-specific transposition mechanism for integration into the bacterial chromosome that superficially resembles the strategy used by some temperate phage when forming prophage (32); there are phage, for example Mu, that replicate through a transposition mechanism (33) and plasmids, such as the F plasmid, that can integrate into the chromosome (34). It is now clear that significant proportions of the genomes of many bacteria are related to mobile genetic elements and have been acquired via lateral transfer.

3. ISLANDS OF HORIZONATALLY-ACQUIRED DNA

Escherichia coli K-12 has played a central role in the development of molecular biology. Once the sequence of its chromosome was deduced in 1997 (35) comparisons with those of related but pathogenic Gram-negative enterobacteria became possible as more and more of their genome sequences became available. These comparisons revealed the presence in the pathogens of large blocks of DNA that E. coli K-12 lacks (36). For example, Salmonella enterica, serovar Typhimurium (S. Typhimurium) has a number of these blocks that contain genes that are essential for invasive disease and intracellular survival (37, 38). Known as 'pathogenicity islands', these clusters of contiguous genes have a higher A+T content than the flanking recipient chromosome, suggesting that they have been acquired horizontally from a source outside the enteric group, and many contain insertion sequences, genes coding for site-specific recombinases and other features that are reminiscent of mobile genetic elements (39-45). Most genomic islands are likely to be former (or even current) mobile genetic elements that have become installed in the genome through the recombination mechanisms related to those used by transposons, phage, integrons or integrating plasmids. It is now clear that very many bacteria apart from E. coli and Salmonella also possess blocks of laterally-acquired DNA that confer new traits (46-54). In the context of the discussion about the relationship between genomic islands and mobile elements, it is interesting to note that transposon Tn*7*, an active mobile genetic element that is widely distributed among bacterial species, has itself been described as forming a genomic island (55).

4. REGULATING THE EXPRESSION OF LATERALLY-ACQUIRED GENES

In many cases, the genes within laterally-acquired islands are subject to complex regulation, often through a combination of controls that involves regulatory genes within the island and those located in the recipient chromosome. How has this complicated control pattern arisen and what is its value? This is a focus of much current research in the field of microbial evolution, and some interesting insights have been gained recently. It is helpful to begin by considering some new data concerning H-NS, a global repressor of transcription that is encoded by a gene in the ancestral chromosome of *Escherichia coli* and related bacteria.

4.1. Repression of horizontally-acquired genes

During 2006, it was discovered that the A+T-rich genes within the pathogenicity islands of *S.* Typhimurium are targeted for repression by the H-NS nucleoid-associated protein (56-58) (Figure 2). This small abundant DNA-binding protein has a preference for binding to A+T-rich DNA sequences and a correlation has been described between H-NS binding sites and intrinsic curvature in DNA (59, 60). Since these features are commonly associated with bacterial promoters (61-63), H-NS can act as a global regulator of gene expression. In all cases where detailed molecular investigations have been carried out, H-NS has been found to act as a transcription repressor (64). The wholesale repression of horizontally-acquired genes by H-NS that has been described in *S.* Typhimurium is now known to occur in other bacteria that express this protein (59, 60). This has led to the attractive hypothesis in which the H-NS protein is proposed to protect the cell from the deleterious consequences of unregulated expression of genes that arrive from outside sources through the processes of lateral transfer (56, 65). In particular, it may be important to store the new genes in an inert state to avoid negative effects on competitive fitness.

H-NS certainly has the properties that one might associate with a protein involved in transcription silencing and the formation of bacterial heterochromatin (66, 67). It is now known to have a preferred (A+T-rich) DNA sequence for high-affinity interaction with DNA and this binding site can serve as a locus from which the protein can oligomerize along the DNA (68). This process has the potential to down regulate, or even completely silence, transcription of many genes. However, the bacterium will require mechanisms to reverse gene silencing if the repressed transcription units are to be expressed for the benefit of the DNA sequences that encode them (by ensuring their transmission to future generations) and the bacterium that houses them (by enhancing its competitive fitness) (Figure 2). It is becoming clear that in the case of H-NS, a variety of mechanisms lead to the containment and/or reversal of its silencing activity.

4.2. Activating H-NS-repressed genes

Studies with the LysR-like protein LeuO show that it has the ability to block H-NS oligomerization along DNA, preserving downstream promoters in an active state (66). The work that established this was conducted in the *ilvIH-leuO-leuABCD* region of the *S.* Typhimurium chromosome where an interesting promoter relay mechanism operates to link the activities of the main promoters of the three resident operons (*ilvIH, leuO,* and *leuABCD*) via changes in local DNA supercoiling (69, 70). However, the LeuO protein has now emerged as a much more general regulator of gene expression that affects several genes where a role for H-NS has been established, including the *hns* gene itself (71-74). LeuO is a particularly interesting example because the protein has such wide-ranging effects on gene expression. It can even influence the expression of the stress and stationary phase sigma factor, RpoS, which is involved in the expression of scores of genes (72, 74). Other H-NS antagonists are somewhat more local in their effects.

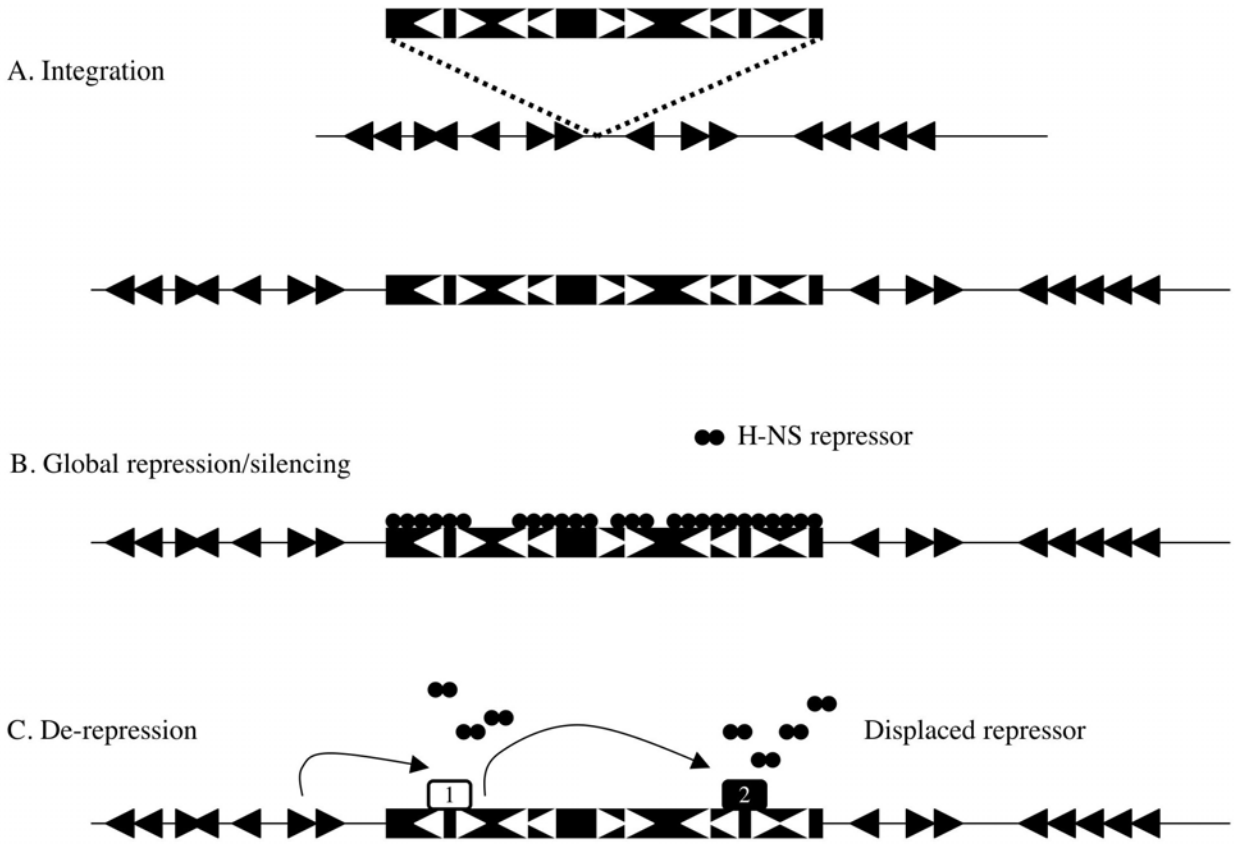

Figure 2. Negative and positive regulation of transcription in a horizontally-acquired genomic island. The incoming island consisting of A+T-rich DNA is represented by the black bar with white arrowheads and this becomes integrated into the chromosome (A). Genes in the integrated island are bound and silenced by the H-NS repressor (B) and silencing is antagonized by anti-repressors (C) that can be encoded by genes in the ancestral genome (1) or by genes located within the genomic island itself (2). Here the anti-repressors are shown displacing H-NS from the DNA. (Not to scale.)

The VirB positive regulator plays a central role in antagonizing H-NS mediated transcription repression in the virulence regulon of the causative agent of bacillary dysentery, *Shigella flexneri* (75-78). These genes are expressed under conditions of temperature, osmolarity and pH that are characteristic of the lower human gut (79). The VirB protein is closely related to plasmid partition proteins of the ParB family and it binds to a sequence that is related to *parS,* the *cis*-acting sites used by ParB proteins to drive plasmid partitioning at cell division, including partitioning of the P1/P7 phage/plasmids (80, 81). VirB works by remodeling the DNA at the target promoter in ways that result in the dislodgement of the H-NS protein. This results in free access to the promoter for RNA polymerase and leads to the initiation of transcription. VirB does not act as a conventional transcription factor because it does not recruit RNA polymerase to the promoter nor does it assist in the isomerization of the closed transcription complex to an open one. Instead it acts as an anti-repressor through its ability to displace the H-NS protein (81).

The SlyA protein is related to MarR-like winged-helix transcription factors (82, 83) and it has a role in opposing H-NS in several Gram-negative bacteria (84-86). In *E. coli,* the SlyA protein acts to regulate the transcription of the *hlyE* haemolysin gene by opposing the repressive activity of H-NS (87). The intracellular concentrations of the SlyA and H-NS proteins play a pivotal role in determining whether the *hlyE* gene is expressed or repressed. This mutual antagonism allows the haemolysin gene to oscillate between active and inactive states depending on the relative abundances of the two regulators (87).

The VirB protein described above is an example of an H-NS antagonist that is encoded by a gene that has been acquired by lateral transfer and that regulates other genes that have been acquired by the same route (88). This is emerging as a common theme among horizontally acquired genes subject to H-NS silencing. Other examples include the VirF and MxiE AraC-like proteins, also involved in positive regulation of virulence gene expression in *S. flexneri* (89, 90), the HilC and HilD AraC-like proteins that up-regulate virulence genes in the SPI1 pathogenicity island of *S.* Typhimurium (91) and SsrB which is a response regulator protein that opposes H-NS repression of transcription in the SPI2 pathogenicity island of *S.* Typhimurium (92). Other examples include the AraC-

like protein ToxT that is involved in up-regulation of H-NS-repressed virulence genes, including the phi-*ctx* bacteriophage-encoded cholera toxin genes, in *Vibrio cholerae* (93, 94) and the MarR-like regulator RovA, a SlyA orthologue that positively controls H-NS-repressed virulence genes in *Yersinia* (85, 95-97).

From the foregoing discussion it can be seen that H-NS-mediated repression of horizontally-acquired genes is emerging as a common feature of many Gram-negative bacteria. It can now be seen that the problem of how to express these repressed genes for the benefit of bacterium has been solved in a variety of ways. Other illustrations of the close relationship between H-NS and horizontally acquired genes include the example of an enzyme encoded by bacteriophage T7 that is specific for the cleavage and inactivation of H-NS (98). Presumably, this is an aid to avoiding wholesale repression of repression of the phage genes following injection of the viral genome into a new bacterial host.

Another example concerns the H-NS paralogue, StpA. This protein is closely-related to H-NS but has a number of distinct properties (99-101). Chief among these is an ability to drive RNA annealing between RNA molecules possessing regions of complementary sequence and an ability to catalyze RNA duplex strand unpairing (102). These activities reveal StpA to be an efficient RNA chaperone. In the context of the present discussion, it is interesting to note that StpA was discovered as a component in the splicing of the *td* intron of bacteriophage T4, itself a horizontally acquired genetic entity (103, 104). In addition to appreciating the influence of H-NS on the expression of genes within laterally-acquired genetic elements, it is interesting to note that several such elements have themselves been found to encode H-NS-like proteins.

5. H-NS-LIKE PROTEINS ENCODED BY HORIZONTALLY-TRANSMITTED GENETIC ELEMENTS

It should also be pointed out that several plasmids capable of self-transmission through conjugation (one of the principal routes for horizontal gene transfer) encode H-NS-like proteins (105, 106). Much of this information has come from bioinformatic analysis; few of the genes coding for such proteins have been examined in detail at the molecular level. One exception is the Ler protein that is encoded by the LEE pathogenicity island in enteropathogenic *E. coli* and another is the Sfh protein that is encoded by the IncHI1self-transmissible plasmid pSf-R27, both of which have now been studied in some depth.

Ler acts to antagonize the negative influence of H-NS on the expression of the virulence genes encoded by the LEE pathogenicity island in enteropathogenic *E. coli* (107-110). Sfh assists the horizontal transfer of the pSf-R27 plasmid by minimizing the impact of the newly arrived plasmid DNA on the H-NS-DNA balance in the recipient a concomitant impact on competitive fitness (111-114). The carriage by mobile elements, or by genetic elements that were probably once mobile, of genes coding for homologues of H-NS that can influence the activity of the host-encoded H-NS protein raises interesting questions about the evolution of gene regulation in the fluid microbial genome. For example it would be interesting to know when these genes were acquired and what difference has their acquisition made to the success the mobile elements in their lifestyle? Might such genes be acquired by other mobile elements in the future, and if so, what impact might this have on the horizontal transmissibility of those elements – for example might it influence the host range of the element by allowing it to enter and replicate in bacteria from which it is currently excluded?

6. PERSPECTIVE

Investigations of gene regulatory matters relating to horizontally-acquired genes are providing important insights into the possibilities for bacteria to expand their repertoire of genetic traits without compromising competitive fitness. Embedding the newly acquired genes in existing gene regulatory circuits is a critical step in the process of integrating new genetic material. Global regulators of transcription are being identified as important contributors to this process, as has been discussed in this article in the case of the H-NS protein. This is a relatively straightforward case because the host-encoded regulatory protein simply silences (or at least down-regulates) the incoming genes, preventing undesirable influences on fitness. The sophistication lies in the methods used by the bacterium to overcome H-NS-mediated repression so that the new genes can be expressed. It is now clear that the cell has a very wide range of options at its disposable. This is an important insight when contemplating routes for intervention in the gene regulatory network to cure or prevent disease, or to manipulate the microbe for some other purpose, such as one related to biotechnology. Information from studies of the regulation of horizontally transferred genes can also inform the development of techniques and the framing of policies aimed at curbing the undesirable consequences of gene dissemination within and between bacterial populations that contribute to the spread of virulence traits and resistance to antimicrobial agents. Although studies of the integration of horizontally transferred genes into host regulatory circuits are still at an early stage of development, it is already becoming clear that bacteria possess remarkable abilities to remodel their gene regulatory circuits to integrate new traits without loss of competitiveness in their environment, showing them to be formidable foes in the global battle for improved human health.

7. ACKNOWLEDGEMENTS

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