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REVIEW PAPER

Phase and antigenic variation mediated by genome modifications

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Abstract Phase and antigenic variation is used by several bacterial species to generate intra-population diversity that increases bacterial fitness and is important in niche adaptation, or to escape host defences. By this adaptive process, bacteria undergo frequent and usually reversible phenotypic changes resulting from genetic or epigenetic alterations at specific genetic loci. Phase variation or phenotypic switch allows the expression of a given phenotype to be switched ON or OFF. Antigenic variation refers to the expression of a number of alternative forms of an antigen on the cell surface, and at a molecular level, shares common features with phase variation mechanisms. This review will focus on phase and antigenic variation mechanisms implying genome modifications, with an emphasis on the diversity of phenotypes regulated by these mechanisms, and the ecological relevance of variant appearance within a given population.

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Introduction

One of the most obvious features of phase variation is the appearance of a minority of colonies or colony sectors displaying a different aspect. Phase variation or phenotypic switch is used by several bacterial species to generate intra-population diversity that increases bacterial fitness and is important in niche adaptation, or to escape host defences. Phase variation allows that the expression of a given phenotype is either ON or OFF; these events are usually reversible (ON $\leftrightarrow$ OFF) but may be irreversible (ON $\rightarrow$ OFF or OFF $\rightarrow$ ON), and result from genetic or epigenetic alterations at specific loci. In contrast to spontaneous mutations, which occur at a frequency of approximately 10^{-8} to 10^{-6} mutations per growing cell per generation, phase variation occurs at frequencies higher than 10^{-5} switches per cell per generation and always affects the same phenotype(s). Phase variation has been described for many different bacterial genera belonging to diverse taxonomic groups and displaying different ecological behaviours (pathogens, saprophytes, symbionts) and can regulated various phenotypes, such as motility, synthesis of pili, expression of capsule, production of antifungal metabolites (Table 1).

Related to phase variation, antigenic variation refers to the expression of a number of alternative forms of an antigen on the cell surface (such as lipoproteins, polysaccharides, type IV pili); this generates within a clonal population individual cells that are antigenetically distinct, allowing bacterial pathogens to escape the host immune system. The term antigenic variation is sometimes used in a broader sense, including the alternative expression (ON ↔ OFF) of genes specifying antigenic identity of a cell surface structure, but here these cases will be classified in phase variation. At the molecular level, some cases of antigenic variation share common features with phase variation mechanisms.

This review will focus on phase and antigenic variation mechanisms implying genome modifications; epigenetic mechanisms such as differential methylation of promoter sequences will not be developed and the reader is referred to other reviews (Henderson et al. 1999; van der Woude and Baumler 2004; van den Broek et al. 2005a). The aims of the present review are: (i) to present an overview of the molecular mechanisms underlying phase and antigenic variation, (ii) to emphasize on the diversity of phenotypes regulated by these mechanisms, and (iii) to discuss the ecological relevance of variant appearance within a given population.

Gene conversion

Gene conversion, highly documented for antigenic variation, involves a recombination event between a silent copy of a gene and another copy that is expressed, and leads to the formation of a new chimeric gene. When several copies of the silent gene are present, numerous chimeric sequences can be theoretically generated, allowing to express various forms of an antigen. There is no common mechanism for gene conversion, and in some cases, proteins of the homologous recombination pathway can be implicated.

Surface proteins in *Borrelia*

Borrelia burgdorferi, the causal agent of Lyme disease, can generate variants expressing different antigenic forms of the VlsE surface lipoprotein. A linear 28 kb-plasmid contains one functional copy of *vlsE* and fifteen silent *vls* cassettes. Segments of the silent *vls* cassettes can recombine with the central region of the *vlsE* gene, generating antigenic diversity in the mammalian host. The donor *vls* cassette and the extremities of the *vslE* gene remain unchanged during this recombination (Zhang and Norris 1998). By this process, *B. burgdorferi* escapes the humoral immune system (Bankhead and Chaconas 2007). Although the precise mechanism underlying this conversion event remains to be elucidated, proteins mediating homologous recombination like RecA are thought to be involved (Liveris et al. 2004). A similar mechanism occurs for antigenic variation of the *Borrelia hermsii* Vmp lipoprotein (Plasterk et al. 1985; Restrepo et al. 1994).

Outer membrane proteins in *Helicobacter pylori*

The outer membrane protein BabA mediates attachment of *H. pylori*, a gastric pathogen, to the Lewis B blood group antigen on gastric epithelium. *H. pylori* cells no longer expressing the BabA protein but expressing the BabB protein appear at a frequency of about 10^{-3}, following a RecA-dependent recombination event between the genes encoding these two proteins (Pride and Blaser 2002; Solnick et al. 2004). Although the function of BabB has not been elucidated, this gene conversion event is thought to provide a control of adhesion allowing to adapt to different niches within the stomach and to escape the host immune system (Solnick et al. 2004).

Pili in *Neisseria gonorrhoeae*

Development of symptoms associated with gonorrhoeae is correlated with the ability of *N. gonorrhoeae* cells to attach and colonize mucous membranes via their pili, despite urinary flux. Various molecular mechanisms, including slipped-strand mispairing, account for the apparition of variants in vivo and in vitro displaying modified surface properties, that are affected in opacity proteins, LPS or pili synthesis (for a review, van der Woude and Baumler 2004). Type IV pili are composed of identical subunits of pilin, a 18-kDa protein encoded by *pilE*. *N. gonorrhoeae* strains carry one or two functional *pilE* loci and four to six *pilS* loci (each locus contains several variable silent copies of *pilS*), lacking promoter sequences and the 5′ portion of the gene (Haas and Meyer 1986;

Table 1 Phase and antigenic variation mechanisms leading to genome modifications

Species[a]	Bacterial class	Ecological niche[b]	Type[c]	Phenotype(s) affected[d]	References
Gene conversion					
*Borrelia burgdorferi**	Spirochaetes	Mammal pathogen	AV	Surface lipoproteins	(Zhang and Norris 1998)
*Borrelia hermsii**	Spirochaetes	Mammal pathogen	AV	Surface lipoproteins	(Plasterk et al. 1985; Restrepo et al. 1994)
Geobacillus stearothermophilus	Bacilli	Soil bacteria	PV	S-Layer proteins	(Scholz et al. 2001)
*Helicobacter pylori**	Epsilonproteobacteria	Human pathogen	AV	Outer membrane proteins	(Pride and Blaser 2002; Solnick et al. 2004)
Mycoplasma genitalium	Mollicutes	Human pathogen	AV	Surface lipoproteins	(Iverson-Cabral et al. 2007)
Mycoplasma synoviae	Mollicutes	Avian pathogen	AV	Surface lipoproteins	(Noormohammadi et al. 2000)
*Neisseria gonorrhoeae**	Betaproteobacteria	Human pathogen	AV	Opacity proteins (Opa)	(Stern et al. 1986)
			AV	Type IV pili	(Howell-Adams and Seifert 2000)
Neisseria meningitidis	Betaproteobacteria	Human pathogen	AV	Opacity proteins (Opa)	(Stern and Meyer 1987)
Site-specific inversion					
*Bacteroides fragilis**	Bacteroidetes	Intestine microflora	AV	**Polysaccharides**	(Krinos et al. 2001; Cerdeño-Tarraga et al. 2005)
*Campylobacter fetus**	Epsilonproteobacteria	Human opportunistic pathogen	AV	Surface proteins	(Dworkin and Blaser 1996)
Dichelobacter nodosus	Gammaproteobacteria	Ovine pathogen	AV	Outer membrane proteins	(Moses et al. 1995)
*Escherichia coli**	Gammaproteobacteria	Intestine microflora	PV	**Type I pili**	(Abraham et al. 1985; Klemm 1986)
Moraxella bovis	Gammaproteobacteria	Bovin pathogen	PV	**Type I pili**	(Marrs et al. 1988; Heinrich and Glasgow 1997)
Moraxella lacunata	Gammaproteobacteria	Human pathogen	PV	**Type I pili**	(Heinrich and Glasgow 1997)
Mycoplasma bovis	Mollicutes	Cattle pathogen	AV	**Surface lipoproteins**	(Lysnyansky et al. 1996; Lysnyansky et al. 2001)
Mycoplasma penetrans	Mollicutes	Human opportunistic pathogen	AV	**Surface lipoproteins**	(Horino et al. 2003)
Mycoplasma pulmonis	Mollicutes	Murine pathogen	AV	**Surface lipoproteins**	(Bhugra et al. 1995)
			PV	**DNA restriction/modification**	(Dybvig and Yu 1994)
*Pseudomonas fluorescens**	Gammaproteobacteria	P.G.P.R.[b]	PV	Root colonization	(Dekkers et al. 1998; Sanchez-Contreras et al. 2002; Martinez-Granero et al. 2005)
Salmonella enterica serovar Typhimurium*	Gammaproteobacteria	Human pathogen	PV	**Flagellin**	(Silverman et al. 1979; Heichman and Johnson 1990)
Insertion–excision					
*Acidithiobacillus ferrooxidans**	Gammaproteobacteria	Soil bacteria (acidic mines)	PV	**Iron oxidation, swarming**	(Schrader and Holmes 1988; Cabrejos et al. 1999)
Citrobacter freundii	Gammaproteobacteria	Human opportunistic pathogen	PV	**Capsule**	(Ou et al. 1988)
*Legionella pneumophila**	Gammaproteobacteria	Human pathogen	PV	**Lipopolysaccharides**	(Lüneberg et al. 1998; Lüneberg et al. 2001)
Neisseria meningitidis	Betaproteobacteria	Human pathogen	PV	Capsule	Hammerschmidt et al. 1996a
*Pseudoalteromonas atlantica**	Gammaproteobacteria	Marine bacterium	PV	**EPS biosynthesis**	(Bartlett et al. 1988; Perkins-Balding et al. 1999)
*Shigella flexneri**	Gammaproteobacteria	Human pathogen	PV	**Cell surface properties**	(Mills et al. 1992)
*Staphylococcus aureus**	Bacilli	Human pathogen	PV	**Biofilm, EPS biosynthesis**	(Kiem et al. 2004)

Table 1 continued

Species[a]	Bacterial class	Ecological niche[b]	Type[c]	Phenotype(s) affected[d]	References
*Staphylococcus epidermidis**	Bacilli	Human pathogen	PV	**Biofilm, EPS biosynthesis**	(Ziebuhr et al. 1997; Conlon et al. 2004)
Xanthomonas oryzae	Gammaproteobacteria	Plant pathogen	PV	Virulence, EPS biosynthesis	(Rajeshwari and Sonti 2000)
Duplication					
Mycoplasma hominis	Actinobacteria	Human pathogen	AV	Adhesins	(Zhang and Wise 1996)
*Pseudomonas tolaasii**	Gammaproteobacteria	Fungi pathogen	PV	**Virulence**	(Grewal et al. 1995; Han et al. 1997)
*Streptococcus pneumoniae**	Bacilli	Human pathogen	PV	**Capsule**	(Waite et al. 2001; Waite et al. 2003)
Deletion					
*Azospirillum lipoferum**	Alphaproteobacteria	P.G.P.R.[b]	PV	Motility and sugar assimilation	(Alexandre and Bally 1999; Vial et al. 2006)
Bordetella bronchiseptica	Betaproteobacteria	Mammal pathogen	PV	Capsule	(Monack et al. 1989)
Coxiella burnetii	Gammaproteobacteria	Human and cattle pathogen	PV	LPS biosynthesis	(Vodkin and Williams 1986; Hoover et al. 2002)
*Haemophilus influenzae**	Gammaproteobacteria	Human pathogen	PV	Capsule	(Hoiseth et al. 1986; Kroll et al. 1988)
*Vibrio cholerae**	Gammaproteobacteria	Human pathogen	PV	Capsule	(Smirnova et al. 1996)
*Yersinia pestis**	Gammaproteobacteria	Human pathogen	PV	Virulence	(Fetherston et al. 1992; Buchrieser et al. 1998)
Slipped-strand mispairing					
Bacillus subtilis	Bacilli	Sol	PV	Swarming	(Kearns et al. 2004)
Bordetella pertussis	Betaproteobacteria	Human pathogen	PV	Fimbriae	(Willems et al. 1990)
			PV	Virulence	(Stibitz et al. 1989)
Campylobacter coli	Epsilonproteobacteria	Human pathogen	PV	**Motility (flagellin)**	(Park et al. 2000)
Campylobacter jejuni	Epsilonproteobacteria	Human pathogen	PV	**Motility (flagellin)**	(Karlyshev et al. 2002; Hendrixson 2006)
			PV	Lipooligosaccharide	(Linton et al. 2000)
*Haemophilus influenza**	Gammaproteobacteria	Human pathogen	PV	Adhesins	(Dawid et al. 1999)
			PV	DNA modification	(De Bolle et al. 2000)
			PV	**Pili (LKP fimbriae)**	(van Ham et al. 1993)
			PV	Hemoglobin receptors	(Cope et al. 2000)
			PV	Lipopolysaccharide	(Weiser et al. 1989; High et al. 1993; Hood et al. 1996)
Haemophilus somnus	Gammaproteobacteria	Cattle pathogen	AV	Lipooligosaccharides	(Inzana et al. 1997; Wu et al. 2000)
Helicobacter pylori	Epsilonproteobacteria	Human pathogen	PV	**Adhesin**	(Backstrom et al. 2004)
			PV	**DNA restriction/modification**	(de Vries et al. 2002)
			AV	Lipopolysaccharides	(Wang et al. 2000)
			PV	**Lysophospholipids**	(Tannaes et al. 2001)
			PV	**Motility (flagellin)**	(Josenhans et al. 2000)
Moraxella catarrhalis	Gammaproteobacteria	Human pathogen	PV	Adhesin	(Lafontaine et al. 2001)
Mycoplasma gallisepticum	Mollicutes	Avian pathogen	PV	Adhesin	(Boguslavsky et al. 2000)
			PV	Lipoprotein	(Glew et al. 1998)
Mycoplasma fermentans	Mollicutes	Human pathogen	PV	**ABC transporter**	(Theiss and Wise 1997)

Table 1 continued

Species[a]	Bacterial class	Ecological niche[b]	Type[c]	Phenotype(s) affected[d]	References
Mycoplasma hyorhinis	Mollicutes	Pig pathogen	AV	**Lipoproteins**	(Yogev et al. 1991)
Mycoplasma mycoides	Mollicutes	Cattle pathogen	PV	**Lipoprotein**	(Persson et al. 2002)
Mycoplasma pulmonis	Mollicutes	Mice and rat pathogen	PV	Surface proteins	(Simmons et al. 2007)
*Neisseria gonorrhoeae**	Betaproteobacteria	Human pathogen	PV	Hemoglobin utilization	(Chen et al. 1998)
			AV	Lipooligosaccharides	(Danaher et al. 1995; Yang and Gotschlich 1996)
			PV	**Opacity proteins (Opa)**	(Stern et al. 1986; Murphy et al. 1989)
			PV	Pilus glycosyl transferase	(Banerjee et al. 2002)
			PV	**Type IV pili**	(Jonsson et al. 1991)
			PV	**Siderophore receptor**	(Carson et al. 2000)
Neisseria meningitidis	Betaproteobacteria	Human pathogen	PV	Capsule	(Hammerschmidt et al. 1996b)
			PV	Hemoglobin receptors	(Lewis et al. 1999)
			AV	Lipooligosaccharides	(Jennings et al. 1999)
			PV	Outer membrane protein (Opc)	(Sarkari et al. 1994)
			AV	Outer membrane protein (PorA)	(van der Ende et al. 2000)
			PV	**Opacity proteins (Opa)**	(Stern and Meyer 1987)
			PV	Pili (addition of phosphorylcholine)	(Warren and Jennings 2003)
			PV	Autotransporter serine protease	(van Ulsen et al. 2006)
*Pseudomonas putida**	Gammaproteobacteria	Soil	PV	Motility	(Segura et al. 2004)
Staphylococcus aureus	Bacilli	Human pathogen	PV	Extracellular matrix-binding proteins	(Buckling et al. 2005)
Streptococcus pneumoniae	Bacilli	Human pathogen	PV	Metabolism and DNA restriction/ modification	(Pericone et al. 2002)
Streptococcus pyogenes	Bacilli	Human pathogen	PV	Surface protein	(Rasmussen and Bjorck 2001)
Vibrio cholerae	Gammaproteobacteria	Human pathogen	PV	Virulence (ToxR regulon)	(Carroll et al. 1997)
Multiple mechanisms affecting a single gene					
*Mycoplasma gallisepticum**	Mollicutes	Avian pathogen	PV	**Adhesins**	(Winner et al. 2003)
*Mycoplasma mycoides**	Mollicutes	Bovine pathogen	AV	**Permease**	(Gaurivaud et al. 2004)
Pseudomonas spp*	Gammaproteobacteria	P.G.P.R.[b]	PV	**Biocontrol traits**	(van den Broek et al. 2003)
*Ralstonia solanacearum**	Betaproteobacteria	Phytopathogen	PV	**Virulence**	(Poussier et al. 2003)

[a] When an asterisk is present, more information about the corresponding species is available in the text

[b] P.G.P.R. = Plant Growth-Promoting Rhizobacteria

[c] Type of variation: AV (antigenic variation); PV (Phase variation)

[d] For phenotypes in bold, a reversion event was reported

Segal et al. 1986) (Fig. 1). Each silent copy of *pilS* can exchange portions of variable regions with a *pilE* locus; in this well-documented conversion event involving proteins of the homologous recombination pathway, the *pilE* locus is altered whereas the *pilS* donor locus remains unchanged (Howell-Adams and Seifert 2000). Pilin antigenic variation exhibits the highest reported frequency of any pathogenic gene conversion system (0.13 recombination events per cell) and can account for the extensive pilin variation detected during human infection (Criss et al. 2005). Among the numerous sequences encoding pilin that

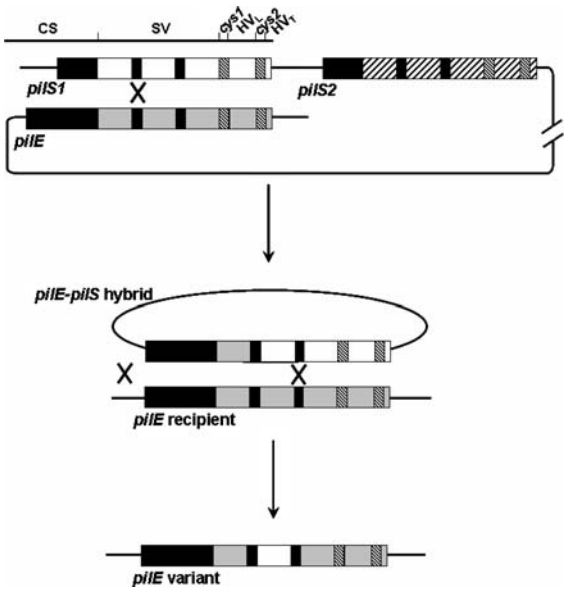


Fig. 1 Pilin antigenic variation in *Neisseria gonorrhoeae* by gene conversion. *pilE* and *pilS* loci are composed of a constant 5′ region (which is shorter for *pilS*), a semi-variable region (SV), a hypervariable loop (HV_L) flanked by two 30-pb conserved sequences, *cys1* and *cys2* and a hypervariable tail (HV_T). DNA exchange occurs between a silent *pilS* locus and the *pilE* locus of the donor chromosome at a short region of homology (small black boxes). The recombination events are indicated by the crosses. The initial model accounting for this gene conversion event, involves duplication of the *pilE* locus following *pilE/pilS* recombination, which would then lead to the excision of a *pilE-pilS* hybrid on a closed-circular piece of DNA. This episome would then recombine with *pilE* via two crossover events, leading to *pilE* gene variation, as depicted here (Howell-Adams and Seifert 2000). A recent finding demonstrating the involvement of the RecBCD recombination pathway suggests that *pilE* gene variation proceeds via a double-chain-break repair model that utilizes the RecBCD enzyme (Hill et al. 2007). Adapted from (Howell-Adams and Seifert 2000)

can be theoretically generated, two particular forms of pilins can appear: the soluble pilin (S pilin) secreted in the extracellular medium originates from the appearance of an amber mutation in *pilE* and the L pilin accumulating within the periplasm originates from the transfer of several silent copies of *pilS* into *pilE*. In both cases, variant cells are non-piliated (Haas et al. 1987). Recombination between *pilS* and *pilE* loci is increased in an iron-deficient medium, suggesting that iron limitation signals for recombinational events triggering pilin antigenic variation, allowing *N. gonorrhoeae* to colonise different sites (Serkin and Seifert 2000). By modifying its antigenic properties, *N. gonorrhoeae* escapes the human immune system and is able to survive in its unique host.

Site-specific inversion

In this process, recombinases recognize short inverted repeat sequences located upstream and downstream of the element to be inverted, an element that usually contains a promoter. The inversion event acts as a switch triggering the expression of a gene initially silent (OFF → IN mode) or impeding the expression of a gene initially expressed (IN → OFF mode).

Polysaccharides and outer membrane proteins in *Bacteroides fragilis*

Genome sequencing of *Bacteroides fragilis*, a bacterium of the intestine microflora that is sometimes pathogen, and several studies evidenced various regions undergoing inversion (Krinos et al. 2001; Cerdeño-Tarraga et al. 2005). At least eight different regions involved in polysaccharides biosynthesis undergo inversion-mediated antigenic variation, allowing *B. fragilis* to synthesize different types of capsules. Mpi, the site-specific recombinase involved in these inversions, not only inverts the promoters of loci involved in polysaccharide biosynthesis but can act on other promoters of genes encoding products of unknown functions (Krinos et al. 2001). Other invertases were evidenced with the genome of *B. fragilis,* some achieving the inversion of promoters related to proteins of the outer membrane (Weinacht et al. 2004; Cerdeño-Tarraga et al. 2005). If DNA

inversion usually controls the expression of a single class of molecules, this mechanism in *B. fragilis* seems to be dedicated to controlling the expression of different classes of molecules, allowing this putative opportunistic pathogen to escape the immune system and to colonize new sites (Cerdeño-Tarraga et al. 2005).

Surface Layer Proteins (SLP) of *Campylobacter fetus*

Campylobacter fetus, an opportunistic human pathogen and the major cause of ovine abortion, can generate variants at a frequency of 10^{-3} affected in the size and antigenicity of SLP. *Campylobacter fetus* possesses eight SLP cassettes, encoding proteins from 97 to 149 kDa. All these cassettes share a 600-pb sequence homology beginning 74 pb before the start codon but only one of the copies (*sapA*) has a functional promoter (Dworkin and Blaser 1996). SLP variation occurs through the inversion of a DNA fragment containing the *sapA* promoter (Fig. 2a); this inversion event moves the *sapA* promoter upstream one of the SLP cassette, allowing the exclusive expression of this cassette. Although the size of the inverted fragment is variable, the frequency of inversion does not seem to be dependent upon the size of the inverted fragment (Dworkin and Blaser 1996). Unlike most of the inversion systems that do not rely on RecA activity, the major inversion pathway of SLP in *C. fetus* is RecA dependent as a *recA*-strain exhibits a reduced frequency of SLP variability; alternative lower-frequency, RecA-independent inversion mechanisms exist (Dworkin and Blaser 1997; Ray et al. 2000). Although SLP appear essential for colonization and/or translocation to the placenta of ewes, they are not required to mediate fetal injury (Grogono-Thomas et al. 2000). SLP switching delays the host antibody response allowing pathogen persistence in an immunologically hostile environment (Grogono-Thomas et al. 2003).

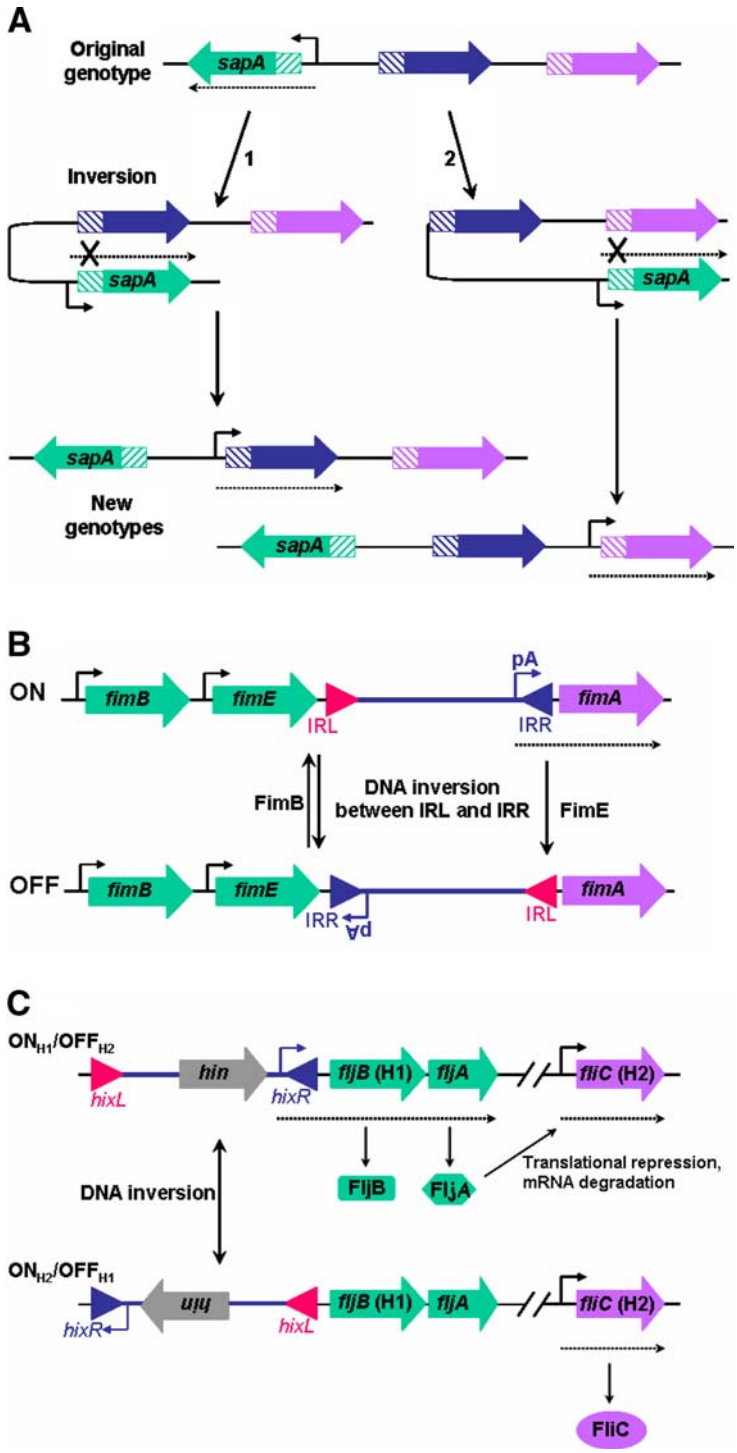
Type I pili of *Escherichia coli*

Escherichia coli can generate, at a frequency of 10^{-3} per cell and per generation, cells lacking pili (Abraham et al. 1985). The mechanism underlying this phenotypic switch relies on the inversion of part of the *fim* operon encoding the type I pili, which are essential for colonization and attachment to eukaryotic cells. The *fimA* gene, encoding the major subunit of type I pili, can either be transcribed (ON position) or be silent (OFF position). Upstream of *fimA* lies an invertible element containing a promoter (Fig. 2b); the inversion of this element modifies the promoter orientation and abolishes *fimA* transcription (OFF position). The inversion is mediated by two site-specific recombinases: FimB and FimE (Klemm 1986). Whereas FimB allows the inversion in both ways, FimE can only mediate the transition from the ON position to the OFF position (Gally et al. 1993). Other regulatory proteins are involved, such as H-NS, Integration Host Factor (IHF), Leucine responsive protein (Lrp), RpoS (Blomfield et al. 1997; Blomfield 2001). pH and osmolarity also influence this invertible switch (Schwan et al. 2002).

Root colonization in *Pseudomonas fluorescens*

While searching for *P. fluorescens* WCS365 mutants deficient in root colonization, the role of a recombinase displaying homologies with site-specific recombinases of the λ integrase family was evidenced. A strain inactivated in this locus (*sss*) colonizes less efficiently than the parental strain the roots of several plants (Dekkers et al. 1998).

During root colonization of alfalfa by *Pseudomonas fluorescens* F113, two types of variants (F and S) with increased motility appear; they preferentially colonize root apex and display unusual long flagella, due to the enhanced synthesis of flagellin. The type F variant also displays an increased synthesis of siderophores but no longer synthesizes antifungal biocontrol compounds such as hydrogen cyanide and exoproteases (Sanchez-Contreras et al. 2002). When alfalfa roots are inoculated with the parental strain, 10% of the root-inoculated cells become variants whereas less than 1% of variants are recovered after inoculation with a *P. fluorescens* F113 *sss* mutant (Sanchez-Contreras et al. 2002). A *P. fluorescens* F113 no longer expressing XerD, a second site-specific recombinase, generates fewer variants than the parental strain (Martinez-Granero et al. 2005). Conversely, strains overexpressing *sss* or *xerD* produce more variants than the wild type in vivo and in vitro.



The *sss* and *xerD* genes are induced by a non-diffusible compound of the plant root (Martinez-Granero et al. 2005). Phase variation could thus play an important role during root colonization and would be mediated by site-specific recombinases induced at the root site.

◀**Fig. 2** Phase and antigenic variation by site-specific inversion. (**a**) Antigenic variation of Surface Layer Proteins (SLP) of *Campylobacter fetus*. The 5′ conserved region and variable regions of the SLP gene cassettes are represented respectively by small stripped box and thick arrows. Only one SLP gene (*sapA*) has a functional promoter (over bent arrow). DNA inversion takes place between two oppositely oriented cassettes flanked by a 6.2-kb DNA element, following DNA exchange within the 5′ conserved region. Inversion of DNA segments containing the *sapA* promoter (over bent arrow) allows expression of alternated SLP gene cassettes (mRNA depicted as thin dashed arrows). Two inversion events and their resulting new genotypes are presented: (1) inversion of the 6.2-kb element alone; (2) inversion of the 6.2-kb element and one SLP gene cassette. For clarity, only three SLP cassettes have been drawn. Adapted from (Dworkin and Blaser 1997). (**b**) Phase variation of type 1 pili in *E. coli*. The relative positions of the promoters (over bent arrows), genes (thick arrows), and inverted repeats IRR and IRL (triangles) are shown. The invertible DNA element is framed by the inverted repeats. IRR and IRL are located within the binding sites for the recombinases FimB and FimE. Binding sites for other regulatory proteins (such as IHF and Lrp) are not shown. Adapted from (van der Woude and Baumler 2004). (**c**) Phase variation of the H1 and H2 flagellins of *Salmonella enterica* serovar Typhimurium. A 955-bp fragment flanked by repeated sequences (*hixL* and *hixR*) gets inverted by the action of the Hin recombinase. This fragment bears the gene encoding the Hin recombinase and a promoter (over bent arrow) allowing the transcription of *fljB* (encoding flagellin H1) and of *fljA* (encoding a negative regulator for *fliC* expression). In the ON_{H1}/OFF_{H2} position, *fljB* is cotranscribed with *fljA*. FljA binds to *fliC* (encoding flagellin H2) mRNA, inhibiting its translation and triggering its degradation. After the inversion event (OFF_{H1}/ON_{H2} position), *fljB* and *fljA* are no longer expressed, allowing *fliC* mRNA to be translated. mRNA are depicted as thin dashed arrows. Adapted from (Yamamoto and Kutsukake 2006)

Flagellins in *Salmonella enterica* serovar Typhimurium

Salmonella enterica serovar Typhimurium cells can express two types of flagellins, H1 or H2, the transition from one serotype to another occurring at a frequency of 10^{-5} to 10^{-3} per cell and per generation (Stocker 1949). A 955-bp fragment bearing a promoter and flanked by repeated sequences (*hixL* and *hixR*) gets inverted by the action of the Hin recombinase, whose gene is located within the inverted fragment (Silverman et al. 1979; Heichman and Johnson 1990). More than a simple transition from an ON position to an OFF position, this system allows the transition from the ON_{H1}/OFF_{H2} position to an ON_{H2}/OFF_{H1} position (Fig. 2c). In the ON_{H1}/OFF_{H2} position, the gene encoding flagellin H1 (*fljB*) is cotranscribed with *fljA* encoding a negative regulator for *fliC* expression. FljA binds to *fliC* mRNA (encoding flagellin H2), inhibiting its translation and triggering its degradation (Bonifield and Hughes 2003; Yamamoto and Kutsukake 2006). After the inversion event, *fljB* and *fljA* are no longer expressed, allowing *fliC* mRNA to be translated (Silverman et al. 1979; Bonifield and Hughes 2003; Yamamoto and Kutsukake 2006). In strain LT2, this inversion can also be mediated by Fin, another DNA invertase located within a resident prophage (Kutsukake et al. 2006). It was recently shown that deletion of *luxS*, a gene responsible for the synthesis of the quorum-sensing signaling molecule autoinducer 2, polarizes flagellar phase variation toward expression of flagellin H1 (Karavolos et al. 2008).

Phase variation of flagellins contributes to virulence in a murine typhoid infection model, as cells locked into expressing *fliC* appear to be more virulent and to survive better in the host than mutant cells locked into expressing *fljB* (Ikeda et al. 2001).

Insertion–excision

Iron oxidation in *Acidithiobacillus ferrooxidans*

Acidithiobacillus ferrooxidans (formerly *Thiobacillus ferrooxidans*) is able to use iron and other sulfur compounds as energy source and is mainly studied for its ability to solubilize metals in mining operations, a process known as biolixiviation. Variants displaying the capacity to spread rapidly on solid medium (LCS "large spreading colony") were observed in vitro on medium containing both ferrous iron and thiosulfate as available energy sources. Those variants have lost the capacity to oxidize Fe^{II} but have retained the ability to oxidize sulphur, and could revert to the parental phenotypes (Schrader and Holmes 1988). The appearance of variants is correlated with the insertion of an IS in *resB* impeding the transcription of *resB* and *resC*, genes involved in the maturation of type C cytochromes essential for Fe^{II} oxidation (Cabrejos et al. 1999). Whether one or several transposition events occur or whether other modifications take place, remains to be determined, as well as the event permitting reversion.

Lipopolysaccharides in *Legionella pneumophila*

Cells of *L. pneumophila,* the causal agent of legionellosis, are entirely covered with lipopolysaccharides (LPS) which synthesis requires a locus composed of 25 genes. From a virulent strain, an avirulent form lacking flagella and LPS was isolated (Lüneberg et al. 1998). During the transition from wild-type to variant, a 30-kb region is excised from the chromosome and replicates like a high-copy number plasmid in the variant (Lüneberg et al. 2001). During reversion, the 30-kb region is inserted back into the chromosome at the initial location. Interestingly, the excised region does not bear any genes involved in LPS biosynthesis but contains ORFs displaying homologies with phage ORFs and genes involved in recombination (*recE*, *recT*, *rusA*). Products encoded by these genes could be responsible of this excision event that is RecA-independant; the excised region is likely to contain a gene encoding a global regulator acting on the different phenotypes altered by phase variation (Lüneberg et al. 2001).

The transition from wild-type to variant as well as the reversion was evidenced in vivo in guinea pig and factors from the host increase the frequency of phase variation (Lüneberg et al. 1998). Variants, although less competitive for infection of the host, could be adapted to the aquatic environment where *L. pneumophila* usually lives (Lüneberg et al. 2001).

EPS synthesis in *Pseudoalteromonas atlantica*

Pseudoalteromonas atlantica is able to colonise various marine environments and several components such as flagella, proteins of the outer membrane, EPS were shown to be involved in *P. atlantica* adhesion. Different colony morphologies can be distinguished on solid medium; type M colonies are large, opaque and smooth, type T colonies are intermediate in size, translucent, shiny and smooth whereas type C colonies are small and wrinkled due to the absence of EPS secretion. Type C colonies are unstable and can revert to types M and T (Bartlett et al. 1988). The mechanism underlying this phenotypic switch relies on the insertion of IS*492* into a gene involved in EPS biosynthesis. A precise excision of IS*492*, occurring at the high frequency of 10^{-3} to 10^{-2} per cell and per generation, was reported allowing reversion to the wild-type phenotype (EPS^{+}). IS*492* belongs to the IS*110* family of atypical insertion elements as no IR sequence can be detected at its extremities, and transposition requires the MooV transposase (Higgins et al. 2007). After excision, IS*492* would remain in the genome in a circular form that may be an intermediate in transposition or a terminal product of excision (Perkins-Balding et al. 1999).

EPS synthesis appears necessary when *P. atlantica* develops biofilms on algae or on sand, but would become dispensable when bacteria go back to the marine environment in the planktonic form (Perkins-Balding et al. 1999).

Cell surface properties in *Shigella flexneri*

Bacteria belonging to the genus *Shigella* are responsible of shigellosis, a dysentery syndrome. Phase variation was evidenced in vitro at a frequency of 10^{-4} per cell per generation in *S. flexneri*. Variants are non-invasive, avirulent, form opaque orange colonies and do no longer express Ipa surface polypeptides. The appearance of variants was correlated with the insertion of IS*1*SFO into *virF*, an invasion plasmid-encoded positive regulator of *ipa* gene expression (Mills et al. 1992). Reversion to a virulent phenotype was observed within human hosts and corresponds to the excision of the insertion element. IS*1*SFO insertion into *virF* is thought to stabilize the invasion plasmid outside the host, a plasmid that is easily cured as virulence genes are not essential for survival outside the host; indeed, opaque variants with essentially unaltered invasion plasmid have a selective advantage compared to individuals with cured or deleted invasion plasmids that can no longer exploit the host environment (Mills et al. 1992).

Biofilms in *Staphylococcus epidermidis/aureus*

The virulence of the human pathogen *S. epidermidis* found on mucous membranes and skin, can be attributed partly to the formation of biofilms. Biofilm development is correlated with the production of the EPS PIA (Polysaccharide Intercellular Adhesin), which synthesis requires the *ica* operon composed of four genes (*icaA–icaD*). From *S. epidermidis* cells able to form a biofilm, variants unable to develop a biofilm can be generated at a frequency of approximately 10^{-5} per cell per generation (Ziebuhr et al.

1997). Wild-type colonies are black on medium containing Congo red whereas small-colony variants are red as they no longer produce the PIA adhesin. An insertion event of IS*256* at different sites within the *ica* operon (predominantly in the *icaC* gene) was evidenced for about 30% of variants (Ziebuhr et al. 1999). Reversion, occurring at a low frequency (below 10^{-8}), is generally accompanied by the precise excision of IS*256* and results in the formation of an episome (Ziebuhr et al. 1999; Loessner et al. 2002). Variants with reduced *ica* expression were associated with IS*256* insertions in *rsbU*, a positive regulator of the stress response regulator σ^B, and in *sarA* encoding a staphylococcal accessory regulator (Conlon et al. 2004). Some strains devoid of IS*256* can generate variants although at a lower frequency, suggesting that other mechanisms could be involved in the appearance of variants in *S. epidermidis* (Conlon et al. 2004). Variants could detach from the biofilm and disseminate into novel habitats. Reversion to the wild-type phenotype could then allow *S. epidermidis* to form biofilms into new environments (Ziebuhr et al. 1999).

In *Staphylococcus aureus*, variants unable to form biofilms also display IS*256* insertions into the *icaC* and *sarA* loci (Kiem et al. 2004). The absence of the σ^B transcription factor dramatically increases the rate of switching to the biofilm-negative phenotype. IS*256*-mediated biofilm switching is reversible, as revertants could emerge from biofilm-negative σ^B mutants (Valle et al. 2007).

Duplication

Virulence in *Pseudomonas tolaasii*

Pseudomonas tolaasii, the causal agent of brown blotch disease of the mushroom *Agaricus bisporus*, degrades fungi tissues by the action of an extracellular toxin, tolaasin, displaying biosurfactant and ion channel-forming properties (Grewal et al. 1995). Aged colonies of *P. tolaasii* often display sectors that when isolated, form distinct colonies; wild-type cells (designated 1116S) show domed, opaque, nonfluorescent colonies and are pathogen whereas variant cells (designated 1116R) present flat, translucent, and fluorescent colonies but are no longer pathogen. Moreover, variants display a stronger chimiotactic response and enhanced motility, and reversion could be observed (Grewal et al. 1995).

The *pheN* regulatory gene, whose deduced product displays homology to both the sensor and regulator domains of the conserved family of two component bacterial sensor regulator proteins, undergoes a 661-bp duplication during phenotypic variation (Han et al. 1997). PheN both acts as an activator for expression of tolaasin and opacity proteins and as a repressor of chemotaxis and of the synthesis of a fluorescent pigment. The duplication event introduces a frame-shift mutation in the predicted *pheN* ORF; this results in the formation of two new non-functional ORFs: a truncated ORF containing only the sensor domain and a second ORF lacking 204 amino acids of the *N*-terminus of PheN and hence the sensor domain (Han et al. 1997). A *recA*$^-$ strain generates 3-fold less variants than the wild-type strain; the few variants obtained from a *recA*$^-$ strain show no duplication within *pheN* but would arise from a punctual mutation in *pheN* (Sinha et al. 2000). Reversion to the wild-type occurs via a precise deletion of the 661-pb region and is RecA-independent as similar frequencies of revertants are observed for 1116R and 1116R*rec*A (Sinha et al. 2000).

Based on differential phenotypic features of 1116S and 1116R, it was proposed that these two forms are adapted to different environmental niches. The wild-type (1116S) would penetrate and proliferate within fungi tissues, inducing their degradation. The variant type (1116R), with increased motility and chemotaxis but no synthesis of virulence factors, would be more adapted to telluric life; by reverting to the 1116S form, the variant would become virulent again and could infest a new host (Grewal et al. 1995). However, the phenotypic switch from the 1116S from to the 1116R form has been demonstrated only in vitro.

Capsule synthesis in *Streptococcus pneumoniae*

Streptococcus pneumoniae is a human pathogen, responsible of otitis, pneumonia and meningitis. Small colonies lacking a capsule can be isolated from a biofilm of *S. pneumoniae* serotype 3 at a frequency of 0.2%. Duplications (from 11 pb up to 239 pb) in the first gene of the capsule biosynthesis pathway (*cap3A*) were evidenced (Waite et al. 2001). Reversion to capsulated cells occurs via the precise excision of the duplication; the frequency of

reversion depends on the length of the duplication as for duplications of 10 pb and 100 pb, excision occurs at a frequency of respectively 10^{-5} and 10^{-3} (Waite et al. 2001). Duplications in other genes of the capsule biosynthesis were evidenced for other serotypes of *S. pneumoniae* (Waite et al. 2003), but the implication of proteins of the homologous recombination pathway, such as RecA, has not been studied.

The ability to regulate capsule biosynthesis is advantageous when invading eukaryotic cells; indeed, adherence and invasion would be 200-fold less efficient for a capsulated strain than for a non-capsulated strain (Ring and Tuomanen 2000). But after cellular invasion, the capsule prevents *S. pneumoniae* from being eliminated by phagocytosis.

Deletion

Motility and sugar assimilation in *Azospirillum*

Azospirillum is a plant growth-promoting rhizobacterium associated with roots of monocots, such as wheat, corn, and rice. *Azospirillum lipoferum* 4B generates in vitro a stable variant, $4V_I$, at frequencies of 10^{-4} to 10^{-3} per cell per generation. Pleiotropic modifications are observed such as the loss of swimming motility, the incapacity to bind some dyes and the inability to assimilate certain sugars (Alexandre and Bally 1999). Interestingly, the frequency of variants generated by a *recA* mutant was increased up to 10-fold, a result contrasting with many studies that showed the abolition or a large reduction of the frequency of phase variation in *recA* mutants (Vial et al. 2004). Recently, the appearance of variants was correlated with the loss of a 750-kb replicon (Vial et al. 2006). *A. lipoferum* 4T, a non-swimming strain displaying all the features of the $4V_I$ variant and retaining the ability to efficiently colonize rice roots, was isolated from the same rice rhizosphere than *A. lipoferum* 4B, suggesting that *A. lipoferum* 4T could in fact be a variant of strain 4B generated within the soil (Alexandre et al. 1996); this non-swimming *A. lipoferum* 4T strain also lacks the 750-kb replicon (Vial et al. 2006). Loss of swimming ability in variant $4V_I$ and in strain 4T is directly linked to enhanced swarming motility (Alexandre et al. 1999). Thus, the non-swimming *Azospirillum* strains are expected to keep the ability to move along plant roots but the adaptive significance of phase variation in *Azospirillum* remains to be established.

Strains of two other *Azospirillum* species, i.e. *A. brasilense* and *A. irakense,* were shown to generate variants, displaying the same phenotypic features as $4V_I$ variant but various stabilities; large-scale genomic rearrangements during phase variation were demonstrated for two additional strains (Vial et al. 2006).

Capsule synthesis in *Haemophilus influenzae*

The type b capsule of the human pathogen *H. influenzae* is a key virulence factor. Loss of capsule at a frequency of 0.1–0.3% was evidenced in vitro and in vivo (Hoiseth et al. 1985). Most of the *H. influenzae* strains possesses an 18-kb tandem duplication of genes involved in type b capsule expression, flanking the *bexA* gene essential for export of capsular polysaccharide. During phase variation, one of these two copies is lost and *bexA* is disrupted, impeding the capsule synthesis (Hoiseth et al. 1986; Kroll et al. 1988). The high-frequency loss of type b capsule expression is probably due to *rec*-dependent recombination between the two copies of the 18-kb tandem repeat, as no deletion occurs in a *recA* mutated strain (Hoiseth et al. 1986).The presence of a capsule would be deleterious for an efficient colonization of epithelial cells. As a duplicated region is more prone to undergo recombination events, the 18-kb tandem repeat could be easily lost when *H. influenzae* no longer needs its capsule.

Capsule synthesis in *Vibrio cholerae*

Vibrio cholerae strain O139, responsible of a cholera outbreak in India and Bangladesh in 1992, expresses various virulence factors such as cholera toxin and capsule, the latter conferring opacity to the colonies. Opaque colonies, after a transfer into liquid medium and a new plating, can generate translucent colonies at a frequency of 3.10^{-3} (Smirnova et al. 1996). The new features of variants, i.e. loss of capsule and purine auxotrophy, are due to the induction of a prophage localized near genes involved in capsule (*cap*) and in purine (*pur*) biosynthesis. The imperfect excision of this prophage would provoke a deletion affecting the *cap* and *pur* genes, and might also affect a gene implicated in regulation of virulence (Smirnova et al. 1996). Variants appear sensitive to serum

bactericidal activity and display decreased expression of virulence factors; as no reversion event was evidenced either in vitro or in vivo, the ecological significance of this phenomenon remains unclear.

Virulence in *Yersinia pestis*

Yersinia pestis, the causal agent of bubonic and pneumonic plague, displays pigmented colonies when grown in agar medium containing haemin or Congo red (Pgm + phenotype). Spontaneous non-pigmented avirulent mutants arise at a frequency of 10^{-5}, with no reversion. Loss of the Pgm + phenotype is accompanied by the spontaneous deletion of a 102-kb region flanked by a repetitive element (Fetherston et al. 1992); this 102-kb region is composed of an iron acquisition segment linked to a pigmentation segment (Buchrieser et al. 1998). Smaller deletions within this island as well as mutation could also cause the non-pigmented phenotype (Buchrieser et al. 1998).

Slipped-strand mispairing

Phase variation may be accomplished via frequent and reversible changes in the lengths of short DNA sequence repeats, generally composed of stretches of polypurines and/or polypyrimidines. The gain or loss of repeat units involves a mechanism of slipped-strand mispairing (SMM), a RecA-dependent process occurring during chromosomal replication, DNA repair and recombination processes that require DNA synthesis. When occurring in the coding sequence of the gene, changes in the number of DNA repeats result in translational frameshift mutations, thereby switching the expression of the encoded protein ON or OFF; gain or loss of repeat units can also affect transcription initiation by modifying the relative positioning of the RNA-polymerase-binding sites within the promoter or transcription termination.

Opacity proteins in *Neisseria*

The opacity proteins (Opa) of *N. gonorrhoeae* and *N. meningitidis*, which mediate bacterial adhesion and invasion of host tissues, undergo both antigenic variation through gene conversion and phase variation through SSM (Stern et al. 1986; Stern and Meyer 1987; Murphy et al. 1989). *Neisseria gonorrhoeae* can host 11–12 *opa* loci, while *N. meningitidis* contains 3–4 loci; bacterial cells either display the Opa^- state, a single Opa protein or express multiple Opa proteins simultaneously. The region encoding the signal sequence of Opa contains a repetitive pentamer sequence (5′-CTCTT-3′), which is subjected to slipped-strand mispairing. Depending on the number of repeats that are present, the translational reading frame of the *opa* gene may be shifted. Hence, each *opa* gene has several ON configurations (i.e. 6, 9, 12 repeats), in which the ATG initiation codon is in frame with the remaining coding sequence; in other configurations (i.e. 4, 8 repeats), the initiation codon is out of frame with the rest of the coding sequence, leading to aberrant or truncated proteins. By altering the expression of individual Opa proteins by SSM and by generating new assortments of Opa proteins by gene conversion, a great variety of combinations with diverse host-receptor specificities can be generated, allowing bacterial populations to adapt to new niches within the same or different hosts.

The expression of other surface components (capsule, outer membrane proteins) as well as other phenotypes can be regulated by SSM in *Neisseria* (Table 1). Moreover, systematic searches of DNA repeats in complete genomes revealed that up to 65 candidate genes can be found in a strain of *N. meningitidis*, showing its high potential for SSM-mediated phase variation (Saunders et al. 2000).

Pili (LKP fimbriae) in *Haemophilus influenza*

One of the virulene factors implicated in the colonization and pathogenesis of *H. influenza* are long thick pili (LKP fimbriae). The expression of *H. influenzae* pili is subjected to reversible phase variation and several observations suggest that expression of pili is beneficial during the early stages of infection but might be disadvantageous in establishing systemic disease. Phase variation is controlled by SSM at the transcriptional level of two divergently orientated genes, *hifA* and *hifB*, encoding respectively the major fimbrial subunit and the fimbrial chaperone. The *hifA* and *hifB* overlapping promoter regions contain repetitive TA units; variation in the number of units changes the normally strictly constrained spacing between the −35 and −10 sequences and results in bidirectional control of transcription initiation (van Ham et al. 1993).

Motility in *Pseudomonas putida*

Frameshift mutations in a poly(G) track at the 5′ region of the *flhB* flagellar gene of *Pseudomonas putida* DOT-T1E are responsible for motility switch. The *flhB* sequence obtained from cells grown on soft-agar plates contain a string of 8 or 11 G's, allowing the synthesis of a functional protein of respectively 380 and 381 amino-acids; when cells are grown in liquid or solid medium, the *flhB* sequences contain 7, 9 or 10 G's, leading to truncated proteins (Segura et al. 2004). These frameshift mutations would allow the cells to save energy under conditions where motility is not necessary. This strain is a toluene-resistant isolate; mutations in genes encoding the flagellar export apparatus, such as *flhB*, lead to hypersensitivity to toluene shocks and growth conditions favoring a functional *flhB* gene (such as growth on soft-agar) result in increased innate tolerance to a sudden toluene shock. So, the presence of intact flagellar machinery is critical for innate solvent tolerance but whether FhlB is directly responsible for solvent tolerance remains to be demonstrated.

Multiple events affecting a single gene

Adhesins in *Mycoplasma gallisepticum/mycoides*

Mycoplasma gallisepticum, an avian pathogen causing respiratory infections in chicken and turkey, can generate variants in vitro at a frequency ranging from 2×10^{-4} to 5×10^{-2} per cell per generation. Those variants have lost the ability to adhere to red blood cells, and no longer express two proteins displaying homology with cytadhesins, GapA and CrmA. Molecular analysis of several variants reveals an amber mutation at the beginning of the *gapA* gene, that also affects expression of the downstream gene, *crmA*. Revertants recovering an intact *gapA* gene can be observed (Winner et al. 2003). In *Mycoplasma mycoides* subsp. *mycoides*, a similar mechanism by an amber mutation was also reported for *ptsG* (a gene encoding a putative glucose PTS permease) (Gaurivaud et al. 2004). Although the occurrence of this phenomenon has not yet been described in vivo, surface protein variation in mycoplasma could be a strategy for host adaptation during the infection.

gacA and *gacS* mutations in *Pseudomonas* spp.

From a collection of *Pseudomonas* strains isolated from the maize rhizophere and displaying antagonist activity towards fungal phytopathogens, the appearance of variants was evidenced in vitro for 43 strains with a frequency ranging from 1.5×10^{-4} to 9.0×10^{-2} depending on the strain (van den Broek et al. 2003). Whereas wild-type colonies are opaque, variants colonies are translucent, do no longer display biocontrol activity, and are unable to produce exoenzymes (lipase, protease, etc.).

In one of these strains, *Pseudomonas* sp. PCL1171, the appearance of variants at a frequency of 6.4×10^{-5} is correlated with point mutation, insertion or deletion events within *gacA* and *gacS*, genes encoding a two-component regulatory system involved in the production of secondary metabolites and exoenzymes in *Pseudomonas* spp. (van den Broek et al. 2003; van den Broek et al. 2005c). Reversion due to recovery of a functional *gacA* or *gacS* gene is observed at a high frequency in vitro; mutants locked in the variant state can be obtained by transposon mutagenesis within *gacS* and complementation with a functional *gacS* allows reversion to the wild-type phenotype (van den Broek et al. 2005c). Moreover, for a *mutS* mutant of strain *Pseudomonas* spp. PCL1171, the frequency of variants appearance is increased by 1,000-fold, probably due to accumulation of mutations within *gacA* and *gacS* (van den Broek et al. 2005b). A strain overexpressing RpoS, the sigma factor involved in stress response, produces ten times more variants than the wild-type strain. RpoS is thought to directly or indirectly repress *mutS* transcription; thus when cells reach stationary phase or when exposed to stress (i.e. when RpoS is expressed), mutations would accumulate within *gacA* and *gacS* (van den Broek et al. 2005b). A *gacS* mutated strain displays a shortened lag phase compared to a wild-type strain. Then, variants would be more competitive and could adapt more easily to the heterogenous and challenging rhizophere ecosystem (van den Broek et al. 2005c).

A common feature between those variants of *Pseudomonas* spp. and variants of *P. fluorescens* F113 mentioned above (in the session "Site-specific inversion") is the presence of mutations within *gacA* or *gacS*; it was thus suggested that *gacA* or *gacS* could be the targets of the site-specific recombinases (Sss and XerD), a hypothesis that remains to be demonstrated (Martinez-Granero et al. 2005).

phcA Mutations in *Ralstonia solanacearum*

Ralstonia solanacearum, the causal agent of bacterial wilting of numerous plants, enters the plant vascular system through natural lesions. Extracellular polysaccharides produced by *R. solanacearum* block xylem vessels, impeding transfer of water and minerals towards the aerial parts of the plant. The expression of numerous virulence factors, including EPS, plant cell-wall degrading enzymes, type-three secretion system, is under the control of the global regulator PhcA. A phenotypic conversion from a mucoid to a non-mucoid state was evidenced in vitro and *in planta*; these non-mucoid EPS^{-} variants display increased motility, reduced endoglucanase activity and reduced virulence but keep the ability to develop *in planta*. Mutations (deletions, duplications, point mutations or IS insertions) within *phcA* are correlated with the appearance of variants from different strains (Brumbley and Denny 1990; Poussier et al. 2003). Reversion to the wild-type phenotype, with recovery of a functional *phcA*, can be observed only *in planta* for variants originating from a 64-pb duplication or an IS insertion event (Poussier et al. 2003).

The avirulent variant would be more adapted to survive in soil, on plant debris or in starving conditions, and the increased motility could allow the colonisation of new niches. When encountering a new host plant, the variant would revert to a virulent state in order to effectively infect a new plant (Poussier et al. 2003).

Conclusions

Historically, phase and antigenic variation, principally investigated in bacterial pathogens of human and animals, was considered to help the bacterium to evade the host immune system. Indeed, many structures that are found to be affected by phase variation are on the cell surface, exposed to the immune system and to the environment (Table 1). However, variation occurs also for genes that are not related to changes in the cell surface properties. Recent studies demonstrated the occurrence of phase variation among plant-associated bacteria whether pathogenic or beneficial, where it affects various phenotypes such as virulence traits, biocontrol traits, root colonization or the ability to form biofilms. Even major metabolic capacities like in *A. ferrooxidans* can be controlled by phase variation, showing the impact of this phenomenon on the ecology of the bacterial species. It is also clear from this survey that phenotypic switch is a widespread strategy used by various bacterial genera belonging to different bacterial classes (Tables 1 and 2).

Phenotypic switch usually affects the synthesis of one family of proteins but several phenotypes can be regulated by this process, like in *B. fragilis* or *A. lipoferum*. For some variants, phase variation could be a strategy to reduce the metabolic load, variant cells avoiding to synthesize factors that are dispensable under certain conditions. Thus, phase variation can be considered as a global strategy used by bacteria to survive environmental conditions or to colonize new environments by creating a heterogeneous population.

Phase variation implying genome modifications can be correlated to various mechanisms, some of which being specific to a bacterial species, such as insertion–excision of a plasmid in *L. pneumophila* (Lüneberg et al. 2001). In the case of point mutations, these can be considered as mutational mechanism occurring at specific loci (often encoding major regulators) at unusually high frequency. The switch to the parental phenotype is not always documented, and whether this is due to the incapacity to revert or to unsuitable conditions for reversion is not elucidated.

Many evidences of phenotypic switch came only from in vitro experiments, questioning the occurrence of this phenomenon under natural conditions and explaining why its functional significance is largely speculative. If epigenetic phase variation is often regulated by environmental factors such as temperature and medium composition, this aspect is less documented for genome modification-mediated phase variation. The putative role of host signals is also scarcely reported (Poussier et al. 2003; Martinez-Granero et al. 2005).

Finally, there are numerous examples of phenotypic switch for which the mechanism remains to be elucidated (Table 2). Indeed, unraveling the phenotypic diversification from microcolony-type biofilms of the emerging opportunistic pathogen *Serratia marcescens* (Koh et al. 2007), the hyperadhesion and enhanced ability to form biofilms of the opportunistic pathogen *Pseudomonas aeruginosa* (Déziel

Table 2 Examples of phase and antigenic variations with uncharacterized mechanisms

Species	Bacterial class	Ecological niche[a]	Type[b]	Phenotype(s) affected[c]	References
Aneurinibacillus migulanus	Bacilli	Soil bacteria	PV	Production of gramicidin S	(Berditsch et al. 2007)
Bartonella henselae	Alphaproteobacteria	Feline and human pathogen	PV	Virulence	(Kyme et al. 2003)
Burkholderia pseudomallei	Betaproteobacteria	Human pathogen	PV	Colony morphology	(Chantratita et al. 2007)
Clostridium chauvei	Clostridia	Ruminants pathogen	PV	**Motility**	(Tamura et al. 1995)
Enterococcus faecalis	Gammaproteobacteria	Human pathogen	PV	**Conjugation**	(Pontius and Clewell 1991)
Mycobacterium avium	Actinobacteria	Human opportunistic pathogen	PV	Motility, antibiotic resistance	(Cangelosi et al. 2001)
Photorhabdus luminescens	Gammaproteobacteria	Symbiont of nematode[c]	PV	Outer membrane proteins	(O'Neill et al. 2002; Joyce and Clarke 2003)
Pseudomonas aeruginosa	Gammaproteobacteria	Human opportunistic pathogen	PV	**Adhesion, biofilm, antibiotic resistance**	(Déziel et al. 2001; Drenkard and Ausubel 2002; Webb et al. 2004; Kirisits et al. 2005)
Pseudomonas brassicacearum	Gammaproteobacteria	Antifungal biocontrol agent	PV	**Motility, production of lipase and protease**	(Chabeaud et al. 2001; Achouak et al. 2004)
Myxococcus xanthus	Deltaproteobacteria	Saprophyte	PV	**Fruiting body development**	(Laue and Gill 1995)
Serratia marcescens	Gammaproteobacteria	Human opportunistic pathogen	PV	Attachment and biofilm	(Koh et al. 2007)
			PV	**Flagellin**	(Paruchuri and Harshey 1987)
Xanthomonas campestris	Gammaproteobacteria	Plant pathogen	PV	**Chemotaxis, xanthan production**	(Kamoun and Kado 1990)
Xenorhabdus bovienii	Gammaproteobacteria	Symbiont of nematode[c]	PV	Virulence	(Pinyon et al. 2000)
Xenorhabdus nematophilus	Gammaproteobacteria	Symbiont of nematode[c]	PV	**Motility**	(Givaudan et al. 1996)
			PV	**Respiratory activity**	(Smigielski et al. 1994)

[a] These bacteria are symbiont of entomopathogen nematodes and contribute to the pathogeny of nematodes

[b] Type of variation: AV (antigenic variation); PV (Phase variation)

[c] For phenotypes in bold, a reversion event was reported

et al. 2001; Webb et al. 2004; Kirisits et al. 2005), and the phenotypic switch affecting response to environmental stress in the causative agent of melioidosis, *Burkholderia pseudomallei* (Chantratita et al. 2007) would also be important medical issues. Of ecological relevance for plant-bacteria interactions is also the higher ability to swim and swarm of *Pseudomonas brassicacearum* variants (Chabeaud et al. 2001; Achouak et al. 2004) and the increased chemotaxis and deficiency in xanthan gum production of the plant pathogen *Xanthomonas campestris* pv *campestris* (Kamoun and Kado 1990).

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