Chapter 5

Genome Instability in Bacteria: Causes and Consequences

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Chapter Outline

1. INTRODUCTION

DNA repair pathways in bacteria are extremely efficient, as the typical mutation rate in *Escherichia coli* cultured under normal conditions is about 2×10^{-10} mutations per base pair (bp) per generation [1]. Nevertheless, various forces can perturb the efficiency or accuracy of DNA repair leading to increased genome instability. This chapter discusses the causes and consequences of some of the best characterized genome instability pathways in bacteria, focusing mostly on *E. coli* and incorporating important findings from other models when possible. From a human health perspective, genome instability is often associated with negative outcomes, such as tumor formation and congenital birth defects; however, from the perspective of a bacterial cell, genome instability can have important positive outcomes, including improved adaptation to host environments, nutrient utilization, and the generation of genetic diversity. Genome instability due to endogenous and exogenous DNA-damaging agents is not discussed here; for more information on this topic, see Chapter 17 and references therein.

2. EFFECTS OF STRESS RESPONSES ON GENOME INSTABILITY

Whether or not prokaryotes possess programmed pathways to modulate mutation rates under stressful conditions remains a controversial topic [2–9]; nevertheless, it is certainly clear (and generally accepted) that several stress responses induce changes in cellular metabolism that can lead to increased genome instability, including the SOS response, the RpoS-regulated general stress response, the stringent response to amino acid starvation, the heat and cold shock responses, and

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the polyphosphate-mediated starvation response. While these responses are discussed separately here, attempts were made to make interconnections since it is clear that these distinctions are largely artificial constructions and that bacterial stress responses are actually an extremely complex, interconnected system that can fine-tune stress responses to a diverse array of different types of stress factors (Fig. 5.1).

In this way, bacteria can monitor threats to genome stability from a similar array of challenges and react accordingly by inducing coordinated and concerted responses to promote genome maintenance.

2.1 The SOS Response

Maintenance of the genome during exposure to DNA-damaging agents is critical for the preservation of species. Unlike higher organisms, bacteria lack sophisticated signaling cascades to regulate cell cycle progression in response to DNA damage; nevertheless, many species have evolved a tightly controlled pathway to minimize the deleterious effects of DNA damage known as the SOS response (reviewed in detail in Ref. [10]). While the specifics of the SOS response vary from organism to organism, a conserved feature is that the primary agonist is single-stranded DNA (ssDNA) coated with the RecA recombinase, a complex that is generally absent under normal conditions. That ssDNA plays this important role should not come as a surprise, as it is a byproduct of many types of DNA damage caused by exogenous sources and is an intermediate in several important DNA repair pathways. It can also be formed through defects in endogenous processes, including chromosome segregation, DNA replication and recombination, maintenance of nucleotide pools, and as a byproduct of harmful metabolic intermediates. The SOS response can also be induced by exposure to environmental conditions that do not directly damage DNA. For example, when starved cells grow on glycerol as the primary carbon source, a cyclic-AMP-dependent pathway can lead to SOS induction [11]. Importantly, exposure to certain antibiotics can also lead to SOS induction via the DpiBA two-component signaling pathway (discussed later) [12] and the SOS response can be involved in the development of antibiotic resistance [13]. Furthermore, the activity of the primary regulator of the SOS response, the LexA repressor, is sensitive to changes in the environment. For example, it becomes unstable in alkaline conditions [14] and it is inactivated in ageing colonies [15] and as cells enter stationary phase [16].

The outcome of activation of the SOS response is a massive transcriptional reprogramming enriched in genes involved in DNA repair, chromosome maintenance, and cell division regulators. During the *E. coli* SOS response, these transcriptional changes include the induction of the genes encoding its two Y-family error-prone DNA polymerases: *dinB*, DNA polymerase IV (Pol IV) and *umuDC*, DNA polymerase V (Pol V) (reviewed in Ref. [17]). The mutagenic potential of these polymerases, particularly Pol IV, is a common feature in several of the pathways discussed in more detail later. The primary function of these polymerases is to insert nucleotides opposite bulky lesions in the DNA, an activity that requires relaxed DNA replication fidelity. Nucleotide incorporation by Pol IV and Pol V is largely error free at their cognate lesions; however, their replication of undamaged DNA can be mutagenic due to improper nucleotide discernment and a lack of proofreading. Mutagenesis by Pol IV and Pol V has been evaluated genetically using a *lac* reporter for mutagenesis in cells with constitutive SOS induction [18]. In this system, loss of

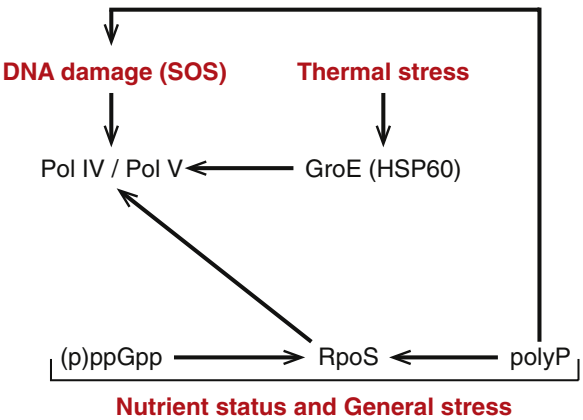

FIGURE 5.1 Interplay between bacteria stress responses that influence genome stability. Bacterial stress responses are not isolated in the cell. As cells respond to different stress stimuli, cross-talk between the pathways leads to tight integration of the input signals and allows the cells to cope with the stress as efficiently as possible via concerted response outputs.

Pol IV reduced the frequency of base substitution mutations by 50–70% and loss of Pol V completely eliminated SOS mutagenesis. Together, these results suggest that while the SOS response functions to stabilize the genome, it can also have potentially mutagenic consequences via the induction of mutator genes.

While the mutagenic outcomes of the SOS-dependent induction of *dinB* and *umuDC* are mostly point mutations [19,20] (although Pol IV can also extend misaligned primers leading to insertions and deletions—see later), SOS induction can also promote higher order genome instability. Integrons are mobile genetic elements (MGEs) that can capture and rearrange open reading frames leading to the formation of novel transcriptional units (operons) controlled by an integron-encoded promoter (reviewed in Ref. [21]). The formation of these new sequences is mediated by an integron-encoded site-specific recombinase, IntI, and through their recombination activities, integrons can be important factors in promoting genome instability. Since the *intI* gene is a target of the SOS response [22], SOS induction can enhance integron activity. Because integrons can be easily transmitted between hosts via mobilization in transmissible elements, such as insertion sequences, transposons, or conjugative plasmids (see later), they can serve as important vectors for the dissemination of genetic information, including antibiotic resistance genes [23]. The SOS response also influences genome stability via effects on other MGEs [24]. For example, SOS induction stimulates the activity of the *Vibrio cholerae* SXT integrative conjugative element (ICE), which can transfer and integrate into recipient genomes carrying with it resistance to several antibiotics [24]. Further discussion of SOS-independent cases of horizontal gene transfer (HGT) follows.

Genome instability leading to the development of antibiotic resistance has also been shown to be a possible consequence of SOS activation by antibiotics, both via DNA damage and independent of DNA damage. In some cases, increased SOS-dependent mutagenesis may accelerate the development of antibiotic resistance [13]. Quinolone antibiotics target two type II topoisomerases that have central functions in DNA replication: DNA gyrase and topoisomerase IV [25]. Disruption of these enzymes by quinolones leads to DNA double-strand breaks (DSBs) that can induce the SOS response [26], thereby increasing the chance that mutations occur in these same genes that lead to antibiotic resistance. In one case, ciprofloxacin treatment promoted the formation of antibiotic-resistant strains of pathogenic *E. coli* via a pathway that required several DNA repair and SOS-regulated proteins: the RecA recombinase, the RecBCD end-processing machinery, and the SOS-induced DNA polymerases II, IV, and V (genetically similar to Cairns–Foster mutagenesis, discussed later). Similarly, deletion of the SOS-regulated DNA polymerase DnaE2 in *Mycobacterium tuberculosis* reduced the virulence of the pathogen and decreased the frequency of mutations conferring rifampicin resistance [27]. While the molecular basis of the reduction of virulence is not entirely clear, one hypothesis is that under normal conditions, the genome destabilizing activity of DnaE2 can promote the accumulation of mutations supporting adaptation to the host immune response and increase the frequency of mutations leading to antibiotic resistance.

β-lactam antibiotics bind to and inactivate penicillin-binding proteins, which are involved in cell wall biosynthesis; thus, these antibiotics are generally only effective in dividing cells. Defects in cell wall synthesis can induce the SOS-response via the two-component signaling system encoded by DpiBA [12]. The SOS-induced gene *sulA (sfiA)* blocks cell division conferring a temporary antibiotic resistance phenotype [12]. The simultaneous increase in the levels of Pol IV and Pol V can then increase the chance of mutations conferring antibiotic resistance. The plausibility of this scenario has been demonstrated in a system in which Pol IV-dependent mutations are significantly increased after exposure to β-lactams [28].

2.2 The RpoS-Mediated General Stress Response

The general stress response, regulated by the alternative RNA polymerase sigma factor RpoS (σ^{38}) in *E. coli* (reviewed in Ref. [29]), can also lead to genome destabilizing outcomes as cells experience nutrient deprivation or other stress. For example, induction of the RpoS regulon increases the expression of the error-prone DNA Pol IV independently from the SOS response [30,31]. Higher Pol IV levels play an important role in the development of mutations in the Cairns–Foster system (discussed later), in which mutations are detected in cells under nutritional deprivation. Entry into stationary phase also modulates the methyl-directed mismatch repair (MMR) pathway (see Chapter X). While MMR is active in stationary phase cells, the levels of two components of the pathway, MutS (the mismatch binding protein) and MutH (the initiating endonuclease), decrease via an RpoS-dependent pathway [32]. Overexpression of MMR proteins in stationary phase cells can suppress mutations [32], suggesting that the decline in MMR after starvation results in a decreased genome stability. While decreased MMR activity cannot cause mutations, it can support the preservation of errors made by other processes as mutations.

Finally, RpoS also influences larger scale genomic rearrangements. For example, under starvation conditions, RpoS is involved in the formation of gene amplifications in the Cairns–Foster system [32]. It is also required for genetic rearrangements that can lead to *araB–lacZ* fusions that occur under carbon-limiting conditions [32]. While other examples exist in the literature, these exemplary cases clearly demonstrate that RpoS can be a potent regulator of genome stability, especially during starvation.

2.3 The Stringent Response

During amino acid deprivation, *E. coli* cells induce a well-characterized adaptive mechanism called the stringent response, which is regulated by the alarmone (p)ppGpp, a multi-phosphorylated guanine derivative [33–35]. During the stringent response, transcription of genes involved in coping with the nutrient limitation is enhanced. Demonstrating the complex intermingling of various stress responses possible in bacteria, the levels of the RpoS sigma factor also increase during the stringent response [32]. In this way, the signals leading to (p)ppGpp production and the general stress response may be coordinated to promote cell survival. As a consequence, the genome maintenance functions of the general stress response may be recruited as part of the stringent response.

More direct connections between (p)ppGpp and genome maintenance have also been reported. In *E. coli* and *Bacillus subtilis*, (p)ppGpp can provide a link between DNA replication and nutrient availability [36]. In *E. coli* (p)ppGpp can block the initiation of DNA replication [37], and in *B. subtilis* it can inhibit replication progression [38].

2.4 Heat and Cold Shock Responses

As bacteria experience shifts in their environment, a central adaptive response is the utilization of alternative sigma factors [56]. As already discussed, the RpoS sigma factor is important for survival of starvation conditions. Two additional sigma factors, RpoH ($\sigma^{32/H}$) and RpoE ($\sigma^{24/E}$), regulate the heat shock response [39]. During exposure to high temperatures, the expression of GroE protein, a highly conserved HSP60 homolog is up-regulated, promoting protein folding and stability during temperature stress and, in some cases, during normal growth [41]. Interestingly, GroE is important for the maintenance of the normal levels of both SOS-induced DNA polymerases, Pol IV and Pol V [32]. Under conditions that reduce the levels of GroE, UV mutagenesis is similarly reduced [32]. This requirement for GroE in stabilizing error-prone polymerases suggests that the heat shock response has some function during heat stress to protect the genome against heat-associated insults. Alternatively, progeny cells may benefit from mutations that could arise as side effects from their activities, suggesting that genome fluidity under such conditions may be advantageous. A less-understood connection also exists between cold shock and genome maintenance. The *E. coli* small histone-like protein HU, which consists of two homologous subunits HUα and HUβ, exists in three dimeric forms: $HU\alpha_2$, $HU\beta_2$, and Huαβ [42]. The cellular composition of these three dimers varies under different stress conditions [43]. While $HU\alpha_2$ and Huαβ seem to be most important under normal conditions, the expression of *hupB*, but not *hupA*, increases during shifts to cold temperatures [32], thus increasing the relative levels of $HU\beta_2$ and HUαβ. A shift to low temperature also robustly induces the expression of the transcription factor NusA [32]. Interestingly, both HUαβ and NusA are required for mutagenesis during starvation in the Cairns–Foster system [32]. Taken together, these observations suggest that both the heat and cold shock responses may modulate genome stability under specific conditions, although the exact biological implications of these potential functions remain unclear.

2.5 Polyphosphate-Mediated Starvation Response

Inorganic polyphosphate (polyP) in *E. coli* is a polymer of orthophosphates that can be tens to hundreds of residues long and are synthesized by polyphosphate kinase (Ppk). While polyP appears to be an energy storage molecule in some organisms, its abundance in *E. coli* seems to be too low to serve such a purpose [44]. Instead, it may function as a gauge for the nutritional status of the bacterial cells. When *E. coli* cells are starved for amino acids, nitrogen, or experience osmotic stress, polyP levels increase due to inhibition of exopolyphosphatase (Ppx), the polyP degradative enzyme and this inhibition depends on increased levels of (p)ppGpp (discussed earlier) [44]. PolyP levels also increase during nutritional downshifts [44] and upon entry into stationary phase and polyP is required for the expression of the *rpoS* gene during stationary phase [45]. In this capacity, polyP may act as an indirect regulator of the repertoire of genes co-regulated in the RpoS regulon and it could serve as rheostat to fine-tune the genome protective capacity of the general stress response under more specific stress conditions.

Quite remarkably, polyP levels also seem to regulate SOS-responsive genes independent of DNA damage [46]. Decreased levels of polyP due to overexpression of *ppx* cause a decrease in DNA damage resistance and block the induction of *recA* and *umuDC* (DNA Pol V)—typical functional markers for SOS activation—after exposure to DNA-damaging agents [32]. Overexpression of *ppk*, which increases the concentration of polyP, induces the expression of *recA* independently of DNA damage or canonical SOS activation by ssDNA-RecA [32]. These observations suggest that the genome protective effects of the SOS response are also recruited in response to stresses unconnected with DNA damage.

3. GENOME INSTABILITY DUE TO STABLE MUTATOR GENOTYPES

Constitutive mutator bacteria have an increased spontaneous mutation rate caused by defects in genes encoding DNA repair factors or other components of other genome monitoring and protective pathways that leads to destabilization of the genome. Of the typical DNA repair pathways (see Chapter 4), the majority of strong mutator phenotypes are due to mutations in the MMR pathway. The MMR pathway is extremely important for maintaining genome stability in *E. coli*, as inactivation of the pathway by mutations in any of the central genes can increase mutation rates between 100- and 200-fold [1]. This large increase in the spontaneous mutation rate in MMR-defective strains reflects the diversity in the types of damage recognized and repaired by this pathway, including incorrectly paired bases (especially due to misincorporation and proofreading failure during DNA replication) and small insertions and deletions. In a 2012 mutation accumulation experiment [1], an MMR-deficient *E. coli* strain had a 138-fold increase in the number of base-pair substitutions compared to the isogenic wild-type strain. The MMR-defective strain also had a 288-fold increase in the formation of insertions and deletions, typically ≤4 nucleotides.

An interesting, and sometimes underappreciated, function of the MMR pathway is to suppress improper recombination [47]. *E. coli* homologous recombination (HR)-mediated by RecA and the RecBCD complex requires perfect or near-perfect homology in the recombining DNA sequences; however, the level of homology required for productive recombination is relaxed in MMR-defective strains. For example, transduction between *Salmonella enterica* serovar Typhimurium and *E. coli* is limited by the recipient's MMR system, which detects and disrupts the formation of heteroduplexes by recognizing sequence divergence [48]. As this example shows, the regulation of recombination by MMR may act as a barrier to HGT between closely related species, thus serving an additional role in preserving genome integrity.

Mutator phenotypes are not due exclusively to defects in the MMR pathway. A key ancillary factor to MMR is the DNA adenine methylase Dam [49]. This protein is required for the DNA methylation that facilitates strand discernment during MMR and has other important roles in DNA replication and gene regulation. Inactivation of *dam* or *drg* (*dam*-replacing genes) in *Pasteurella multocida* leads to robust mutator phenotypes [50]. Mutations in *dnaQ*, which encodes the proofreading (epsilon) subunit of the replicative DNA polymerase III, cause remarkable increases in mutation rate due to defective removal of misincorporated nucleotides during DNA replication. Mutations in genes encoding the GO system (*mutM*, *mutY*, and *mutT*), which repairs oxidized guanines (8-oxodG), lead to medium to high mutator phenotypes (eg, see Ref. [51]). Other mutator genes encode proteins that prevent DNA damage via detoxification, rather than repair damages, such as *oxyR* [52] and *sodA* [53].

Mutator strains are estimated to make up to about 1% of the natural *E. coli* population, and mutator phenotypes occur in both commensal and pathogenic strains [54]. Constitutive mutators represent a powerful challenge for the medical field as they tend to be common in infectious diseases, such as cystic fibrosis (discussed here), urinary tract infections [55], and food-related diseases [56]. This section focuses on one of the best-characterized examples of the impact of mutator strains on clinical practice: *Pseudomonas aeruginosa* colonization of cystic fibrosis (CF) patients.

CF is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) and is the most common autosomal-recessive genetic diseases in Caucasians [57]. The physical effects of CF lead to a strong predisposition for chronic respiratory infections (CRIs), which are the main cause of high morbidity and premature mortality in CF patients [58]. While infections with a range of bacteria can occur in CF patients, including *Haemophilus influenzae* and *Staphylococcus aureus*, *P. aeruginosa* infection is the most common [59]. Quite remarkably, an early study revealed that up to 20% of the isolates from 37% of the patients examined were chronically colonized by mutator strains of *P. aeruginosa* [60]. In contrast, patients presenting with acute infections did not show such enrichment for mutators [61]. These observations suggest that mutators were especially associated with CRIs. This concept was confirmed by a later study that showed that the proportion of mutator isolates increased from 0% to 65% after 20 years of chronic infection [62]. The genetic basis for these mutator phenotypes was, as might be expected, largely due to defects in the MMR pathway, as between 60% and 90% of the mutator isolates had mutations in MMR genes, most commonly *mutS* [63–65].

One intensely studied consequence of CRI by mutator strains of *P. aeruginosa* is the development of antibiotic resistance. After many years of aggressive antibiotic treatment, antibiotic resistance is more common in *P. aeruginosa* strains isolated from chronically infected CF patients compared to isolates from acute infections [66]. Oliver et al. (2000) first characterized the prevalence of mutator strains among antibiotic-resistant *P. aeruginosa* strains isolated from CF patients. They demonstrated that mutator strains were more frequently resistant to eight commonly used therapeutics against *P. aeruginosa* infection: up to 40% of mutators were resistant compared to only 5% of nonmutators. This correlation between mutator phenotypes and antibiotic resistance were subsequently corroborated by follow-up studies [62,67]. In one particularly interesting study, Ferroni et al. (2009) demonstrated that mutator strains acquired additional antibiotic resistance more rapidly than nonmutator strains. The role of mutator genotypes in promoting antibiotic resistance is not unique to *P. aeruginosa*, as similar relationships have been found in *S. aureus* and *H. influenzae* strains isolated from CF patients [68,69].

A CF patient is normally colonized by a single strain of *P. aeruginosa* that persists through their lifetime [70]. The host respiratory tract represents a microenvironment in which different selective pressures can act on the strain leading to the divergence and fixation of phenotypic variants. In many cases, these novel phenotypes are due to loss-of-function mutations that contribute to host adaptation and support chronic infection. For example, mutations that attenuate virulence genes may shift the pathological outcomes of the infection away from acute damage and toward chronic effects that support persistence. The genetic underpinning of such adaptation has been temporally characterized using whole-genome sequencing [71]. Comparison of the genomes from early and late isolates revealed the accumulation of up to 68 mutations that, in many cases, resulted in the loss of function of virulence genes, representing a virulence adaptation that favored long-term colonization (as mentioned earlier). Not surprisingly, subsequent work demonstrated that this rapid genetic adaptation was driven by a mutator phenotype [63].

This example demonstrates that constitutively higher mutation rates and the corresponding increase in genome instability can be beneficial to an organism as it faces the challenges of its environment; however, such genomic fluidity can also have detrimental outcomes for the organism. For example, adapted strains of *P. aeruginosa* isolated from CF patients have reduced transmissibility [72]. Furthermore, while nonmutator strains can spread between CF patients, the spread of mutator strains has not been observed [65]. Finally, highly adapted mutator isolates have decreased fitness and virulence in secondary environments [73]. Thus, it is clear that, in fact, the genome instability induced by mutator genotypes results in a tug-of-war between potentially beneficial and detrimental outcomes, which are sorted out based on the selective pressures of the environment. While this consequence of genome instability is specifically demonstrated in this example, it likely represents a universal attribute of organisms with elevated mutation rates.

4. GENOME INSTABILITY DUE TO HOMOLOGOUS AND ILLEGITIMATE RECOMBINATION

The genomes of bacteria carry sequences that are especially prone to participate in recombination transactions. Depending on the context, such recombination can lead to intrinsic genomic instability. This section focuses on just a few well-studied examples with important biological outcomes, especially for adaptation and virulence.

4.1 Microsatellite Instability

Microsatellites, or simple sequence repeats (SSRs), consist of repetitions of sequences less than 5 or 6 bps. In bacteria, these sequences are unstable and can expand and contract and have higher spontaneous mutation rates and can thus be potent drivers of adaptation [74]. While the causes of this instability are varied, illegitimate recombination due to strand slippage is a driving factor. Depending on the location in the genome, expansion and contraction of microsatellites can have varying phenotypic outcomes. Contingency loci in bacteria are unstable genetic sequences that can lead to heritable genotypic switching [75]. In some cases, this genetic instability is due to the presence of microsatellite DNA, whose length can vary leading to changes in gene expression and sometimes phase variation. Phase variation is a programmed alteration of the genome that leads to heritable and reversible phenotypic outcomes at frequencies higher than the background spontaneous mutation rate [76]. These phenotypic changes are often important for adaptation to specific niches, including colonization of hosts by pathogens, and survival in new environments. Fimbrial expression in the opportunistic pathogen *H. influenzae* presents one of the best-characterized examples of microsatellite-mediated phase variations (Fig. 5.2A) [77]. *Haemophilus influenzae* cells exhibit different levels of fimbriae expression depending on their biological niche: nasopharyngeal isolates tend to be fimbriated, while isolates from systemic infections are not. Fimbrial phase transitions depend on the differential regulation of the *hifA* and *hifB* genes, which encode the fimbrial structural protein and chaperone, respectively. This switch in transcriptional activity depends on the number of dinucleotide TA repeats between the −10 and −35 sigma factor recognition sequences in the overlapping promoters of *hifA* and *hifB*. Expansions and contractions in the number of TA repeats due to slipped strand mispairing determine whether the spacing of the −10 and −35 sequences in each gene's promoter are optimal for binding by RNA polymerase.

4.2 Gene Conversion

Gene conversion occurs when two related but divergent sequences exist in the same cell and can be substrates for recombination [78]. The outcome of gene conversion is a unidirectional transfer of genetic sequence information from a donor sequence into a highly similar recipient sequence. One of the most studied outcomes of gene conversion in bacteria is the type IV pilus antigenic variation in *Neisseria gonorrhoeae* [79]. Antigenic variation leads to phenotypic heterogeneity within a genetically clonal bacterial population, as different cells can express one of a several possible antigenic forms

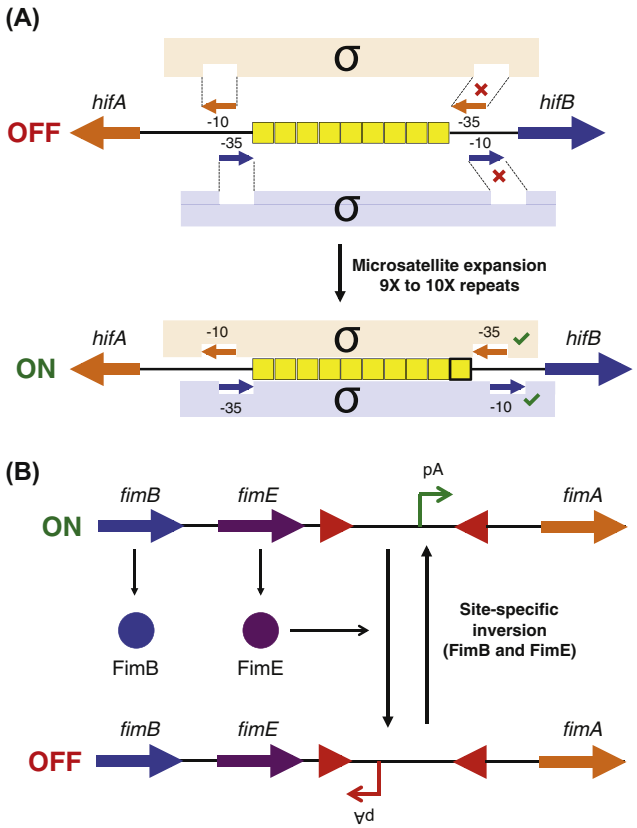

FIGURE 5.2 **Recombination-mediated genome instability leads to changes in gene expression.** Bacteria have harnessed genome instability mediated by specialized DNA sequences to tightly control gene expression. (A) Microsatellite expansions and contractions in *Haemophilus influenzae* regulate the expression of the pilus by altering the spacing between the −10 and −35 promoter elements. Changes in this spacing control the binding ability of the RNA polymerase sigma factors. Different numbers of TA repeats (*yellow boxes*) either block expression (9X repeats), lead to optimal expression (10X repeats), or promote limited expression (11X repeats). (B) Site-specific inversion controls the expression of fimbriae in *Escherichia coli* by switching the orientation of the promoter for the structural gene for type-1 fimbrial protein, *fimA*. This switching, mediated by the FimB and FimE recombinases (encoded by adjacent genes), leads to an off–on switch in gene expression.

of a protein. In *N. gonorrheae*, the antigenic pilus variants are due to the differences in the structure of the pilin protein. Each pilin variant shares a conserved N-terminal region but they differ in the C terminus. The conserved part of pilin is encoded by the *pilE* locus and the variable segment of the protein is encoded by up to six different nontranscribed or weakly transcribed *pilS* loci [80,81]. The expression of a full length, functional pilin protein requires a gene conversion event in which one of the silent *pilS* loci is transferred to the *pilE* locus via a recombination reaction mediated by RecA and RecOR. While the *pilE* locus is genetically unstable, the gene conversion event has no effect on the *pilS* loci. The biological forces driving pilin antigenic variation in *N. gonorrhoeae* are not fully understood; however, iron levels may influence the frequency of antigenic variation and it may be important during transfer into new hosts [82].

4.3 Site-Specific Inversion Systems

A common feature in bacterial genomes are site-specific inversion systems [83–85]. These genetic features range in size from about 100 bps to 35 kilobase pairs (kbps) and are flanked by two terminal inverted repeats. At a frequency of between 10^{-3} and 10^{-5} per cell per generation, these repeats are recognized by an invertase (a specialized recombinase), encoded either in the fragment that is inverted, or elsewhere in the genome, and undergo an inversion event. In general, the inversions result in an on-off toggle and can have a variety of biological consequences, especially in regulating the production of proteins that form structures on the surfaces of cells (eg, flagella and pili). Site-specific inversion at the *fim* locus is the underlying mechanism for fimbrial phase variation in *E. coli* [86–88], which can be an important determinant of virulence in uropathogenic *E. coli* strains (Fig. 5.2B) [89–93]. In this system, the *fimA* gene, which encodes the fimbrial structural protein, is under the control of a σ^{70} promoter that lies on the inverting fragment. The FimE and FimB invertases can mediate

the inversion of this fragment, placing the promoter in either the on or the off position, depending on the orientation. This inversion system depends on several *E. coli* small histone-like proteins, such as H-NS and IHF [94,95] and can be influenced by environmental conditions, including changes in nutrient availability [96] and exposure to human urine [97]. The stress factor (p)ppGpp also influences the frequency of inversion [98], demonstrating yet another interaction between stress signaling and genome instability. Site-specific inversion represents an example in which the bacterial cell has exploited genomic instability to integrate information from complex environmental signals to finely regulate gene expression.

4.4 Error-Prone Double-Strand Break Repair

In most normal circumstances, the repair of DNA DSBs is considered an error-free repair process, since an undamaged homologous molecule is used as a template for the repair; however, a collection of work has revealed that under stressful conditions bacteria can switch from a high-fidelity DSB repair pathway to a lower fidelity pathway. This switch to low-fidelity repair has been most extensively worked out in the Cairns–Foster adaptive mutation Lac reversion assay [99,100]. This system was mostly used in *E. coli*, but it was also adapted for use in *S. enterica*, and while the features are generally similar in both species, certain divergent features have been reported [9]. This discussion focuses on the *E. coli* model, as it has been more extensively analyzed.

In the Cairns–Foster system, a Lac^- *E. coli* strain carrying a +1 bp frameshift in a *lac* allele on an F′ conjugative plasmid is grown in minimal medium with glycerol (or another nonlactose carbon source) to stationary phase. During this growth period, typical Luria-Delbrück spontaneous, selection-independent mutations occur. These stationary phase cells are then plated on minimal medium containing lactose as the sole carbon source so that only cells that revert to Lac^+ can form colonies. Over the course of several days, Lac^+ colonies form continuously with more or less linear kinetics. Extensive analysis of the genetic requirements for this reversion under selection has revealed that several stress responses are required: the RpoS general stress response, the SOS response, and the RpoE envelope stress response (reviewed in Ref. [32]). During the first 5 days of incubation, most colonies have a compensatory −1 bp frameshift in a homopolymeric sequence of guanine residues [32]. In subsequent days, colonies containing amplifications of the *lac* allele become the predominant type [32]. While the point mutations and the amplifications happen independently [32], it is clear that both are stimulated by stress since the RpoS response is required in both cases. Analysis of the molecular requirements for the point mutations has revealed that proteins involved in DSB repair via HR are central to the process [32]. While dissenting opinions do exist [9], it is generally accepted that the stress-induced point mutations are due to an RpoS-mediated switch from high-fidelity HR-dependent DSB repair to a low-fidelity mode of HR-dependent DSB repair at a persistently generated DSB [101,102]. When cells are growing normally without stress, DSB repair by HR is a multistep process that ends with the synthesis of homologous DNA by the high-fidelity replicative DNA polymerase III (Pol III) (see Chapter X for a review of this pathway). During starvation, and upon activation of the RpoS response, it is thought that the SOS and RpoS-regulated error-prone DNA Pol IV (see the preceding paragraphs) replaces Pol III leading to a switch to mutagenic DSB repair [30,32]. Via its ability to extend misaligned primers, DNA synthesis by Pol IV is the main source of the −1 frameshifts in the mutant *lac* allele. It is important to bear in mind that while certain models for the formation of these point mutations require the *lac* allele to be carried on the F′ plasmid and invoke selection as the driving force (rather than a stress-induced increase in the mutation rate) [9], ongoing research has largely ruled out that the F′ plasmid is an essential component of stress-induced mutations. It is now clear that Cairns–Foster mutagenesis can also occur in starved cells with no plasmid and in vastly different, *lac*-independent experimental systems (eg, a tetracycline resistance reversion assay) [101,102]; thus, it is likely that this mutational pathway is not a phenomenon specific to the original Lac reversion system, and that it likely has broad implications for genome stability under stressful conditions.

Since its discovery, research on Cairns–Foster mutagenesis has largely focused on unraveling the complex basic genetic requirements of the pathway in *E. coli* and *S. enterica*, without much emphasis on the implications of the pathway. Interestingly, some findings hint that Cairns–Foster mutagenesis may have important clinical consequences. As discussed earlier, exposure to ciprofloxacin causes resistance to the drug via a mutagenic pathway that shares most genetic requirements with the low-fidelity DSB repair underlying Cairns–Foster mutagenesis [13]. In another example, exposure of pathogenic *Salmonella* to bile can lead to accumulation of mutations that confer bile resistance [103]. Quite remarkably, this mutagenic pathway also requires DSB repair proteins, Pol IV, and SOS proteins. These examples suggest that stress-induced Cairns–Foster mutagenesis may have important implications in understanding the evolutionary forces driving the development of antibiotic resistance and may provide new targets for drugs to slow the formation of novel antibiotic-resistant pathogenic strains. As our ability to dissect complex molecular mechanisms continues to improve, it is critical that we are willing to sever ties to or to refine classical models to avoid missing such potentially important biological processes.

5. GENOME INSTABILITY DUE TO SPECIALIZED GENETIC ELEMENTS

Despite the relative simplicity of bacteria, their genomes are extraordinarily complex and consist of a number of functional elements in addition to the core coding genes and their respective basic regulatory sequences. Many of these ancillary elements have properties that allow them to move around in the genome and these special sequences are called mobile elements. In many cases, the movement of mobile elements within the genome can have mutagenic outcomes and, thus, they represent an important threat to genome stability. Mobile elements are very diverse and, for the sake of brevity, only four types of intrinsic elements are considered here: insertion sequences, transposons, integrons (discussed with the SOS response earlier), and miniature inverted-repeat transposable elements (MITEs) (Fig. 5.3).

The current discussion focuses on intrinsic genome instability caused by mobile elements. Genome alterations via transmissible mobile elements between cells are discussed in the following section.

5.1 Insertion Sequences

Insertion sequences are small (<2.5 kb) DNA segments delimited by short terminal inverted repeats that contain one (or sometimes two) open reading frames that encode proteins specifically required for the mobility of the insertion sequence, that is, a transposase [104]. Insertion sequences can represent a severe threat to genome instability as their insertion always changes the bacterial chromosome; however, excision can either restore the original sequence of a chromosome, or generate a mutation. Insertion results in the introduction of foreign DNA sequence (the transposase gene) and often the molecular exchanges leading to insertion result in the formation of direct repeats. Insertion can interrupt a gene or can alter genetic regulatory sequences leading to changes in gene expression or even inactivation of genes. Improper excision of insertion sequences can either leave some insertion sequences behind, resulting in an insertion, or remove some host DNA, resulting in a deletion. Recombination between insertion sequences and homologous DNA in the host can also lead to genomic rearrangements. Despite their small size and relative simplicity, the biology of insertion sequences is complex.

The genomic instability resulting from insertion and excision of insertion sequences can have important consequences for bacterial cells, including some pathogens. One biological outcome of insertion sequence mobility is phase variation (introduced earlier). Insertion of IS*492* in the *eps* locus of *Pseudoalteromonas atlantica*, a pathogen of crabs, prevents expression of extracellular proteins involved in biofilm formation [105,106]. Precise excision,

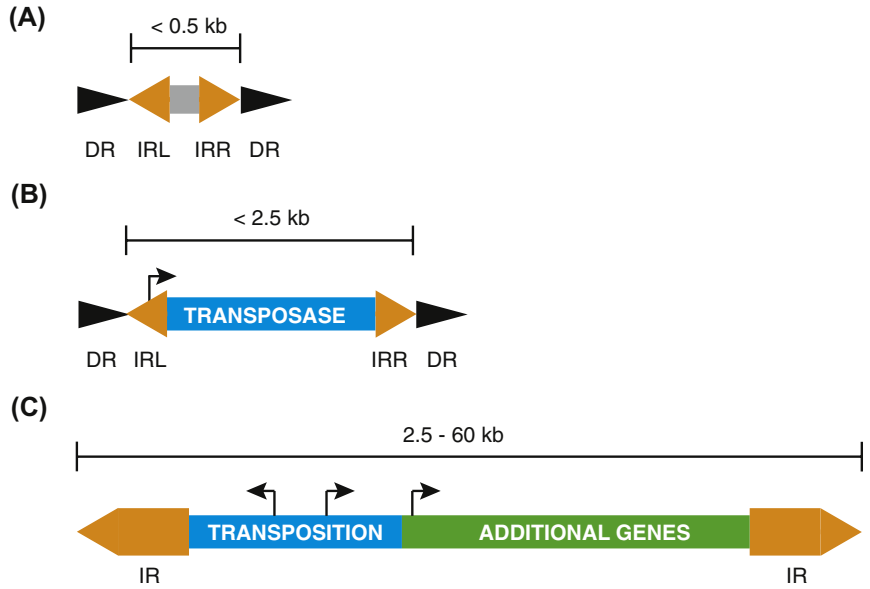

FIGURE 5.3 Typical structures of mobile genetic elements. (A) MITEs are small elements delineated by inverted repeats (IRL and IRR) and bordered by direct repeats that are formed during insertion (DR). MITEs do not encode additional genes. (B) Insertion sequences are larger elements that, like MITEs, are also flanked by direct repeats and inverted repeats. Unlike MITEs, insertion sequences encode at least one gene for a transposase. (C) Transposons have more complex structures and are much larger than MITEs and insertion sequences. They are flanked by long inverted repeats and typically encode several genes. One gene cassette encodes several genes required for transposition (blue) and the other encodes additional genes with various functions, especially common are genes that confer drug resistance (green).

mediated by the transposase MooV, produces a circular insertion sequence-derived molecule and allows expression of the *eps* locus [107,108]. In this case, genomic instability introduced by the insertion sequence offers the bacterial cells phenotypic variability to adjust to different environmental conditions.

5.2 Transposons (Nonconjugative)

Transposons are similar to insertion sequences except that they encode additional factors independent of their mobility functions, including genes for antibiotic resistance, virulence, and fitness (eg, heavy metal resistance and enhanced metabolic capabilities). As a consequence, transposons tend to be much larger, ranging in size from 2.5 to 60 kb, and are flanked by terminal repeats. For the most part, the genomic instability induced by transposons is similar to that caused by insertion sequences, that is, gene disruption or deregulation and larger scale genome alterations, such as deletions, duplications, and inversions within one cell. These alterations depend on the mobilization of transposons; thus, the root of transposon-induced genome instability is how the transposon activity is regulated. Any stimulus that induces transposon activity, even if it does not cause DNA damage itself, can induce genome instability secondarily in cells that carry certain transposons. Such stimuli are diverse and range from intrinsically regulated factors, to regulation by host elements [109–111].

Because transposons can carry large amount of genetic information, their movement can have remarkable biological consequences. Two well-known complex transposons from *E. coli* are particularly well-known vectors for antibiotic resistance: Tn*10* [112–114] encodes tetracycline resistance and Tn*5* [115,116] encodes resistance to kanamycin, bleomycin, and streptomycin. These transposons are more complex as they are flanked by insertion sequences (IS*10* and IS*50*, respectively) and can integrate into chromosomes from divergent bacterial species. Some even more complex transposon-like sequences, often called ICEs, encode conjugation functions and are discussed in the following section.

5.3 Miniature Inverted-Repeat Transposable Elements

MITEs are short AT-rich sequences (<0.5 kb) that contain terminal inverted-repeat sequences and, in many cases, lie within a stretch of target site duplications [104]. Some of the first MITEs were the Correia elements of *N. gonorrhoeae* and *Neisseria meningitides* [117,118] and they are widespread in eukaryotic genomes, but in 2011 only, their distribution in bacterial genomes was fully appreciated. Depending on the model system, MITEs are also known by alternative terms, including RU elements (enterobacteria) and RUP, BOX, or SPRITE elements (*Streptococcus*) (for details, see Ref. [119]). While similar to transposons, a distinctive feature of MITEs is that they do not encode a transposase; however, they can commandeer transposases from other mobile elements for mobilization [120,121].

MITEs can induce several types of genome instability, including the introduction of genetic material, gene inactivation, changes in gene regulation, or even deletions and chromosomal rearrangements [120]. Studies on Correia elements have provided some particularly clear and interesting outcomes of their activity. Correia insertion points are hotspots for recombination and rearrangement [122,123] and they can alter the stability of the mRNA from neighboring genes [124,125] or act as transcriptional terminators [126]. Quite remarkably, Correia elements also have a −35 sequence compatible with the vegetative σ^{70} RNA polymerase appropriately positioned with a TATA sequence [127]; thus, they can form ectopic promoters at their insertion sites. In fact, Correia elements have been shown to control the transcription of several genes in *Neisseria* species. As work continues on bacterial MITEs, more consequences of their genome destabilizing properties are sure to emerge.

6. GENOME INSTABILITY DUE TO GENETIC EXCHANGE

Bacterial genomes have a remarkable ability to accommodate foreign DNA, either from related strains or even highly divergent species, which can confer a selective advantage to the organism. This process of sharing of relatively large pieces of genetic information is called HGT and it is mediated by MGEs. HGT commonly occurs by three mechanisms: transduction, conjugation, and transformation. As discussed earlier, the nature of MGEs is diverse and they differ in their specific molecular properties [109]. Common MGEs that are subject to transmission include plasmids, bacteriophages, pathogenicity islands (PAIs), insertion sequences and transposons (discussed earlier), and the broad class of ICEs, which includes some conjugative transposons. While bacteria have mechanisms to protect their genomes against invasion and modification by MGEs, the potential advantages of acquiring foreign DNA has certainly led to the evolution of some flexibility in these systems to take advantage of the potential benefits of the genome destabilizing effects of HGT. Diverse species of bacteria share a core set of mechanisms for the dissemination and sharing of genetic information: transduction, conjugation, and transformation. Through these different processes, DNA can be directly shared between donor and recipient cells

(conjugation) or can be transmitted via cell-independent mechanisms (transduction and transformation). The outcome of each process for the recipient cells is a genomic expansion via the acquisition of often nonessential genetic material.

6.1 Transduction

Transduction is a process mediated by bacterial viruses called bacteriophages (or phages) in which they transfer DNA by an infectious process. Transduction comes in two basic flavors: generalized transduction and specialized transduction. While the precise processes of generalized and specialized transduction differ, the end outcome is the same: host DNA is inadvertently packaged into phage particles. Upon subsequent rounds of infection, this DNA is then injected into the recipient cell leading not to a productive infection, but to the exchange of nonphage genetic material. Phage can carry virulence factors as part of their core genomes that can be expressed under specific conditions, such as the transition from a *lysogenic phase*, when they are integrated into the host chromosome, to the *lytic stage*, when they actively replicate for reinfection [128]. HGT by phages can lead to the conversion of a nonvirulent bacterial strain into a virulent strain when phage-borne genes are delivered into potential proto-pathogens. The potential complexity of phage-mediated virulence acquisition is exemplified by the relationship between *V. cholerae* and its phage CTXϕ (Fig. 5.4) [129].

The primary virulence factors of *V. cholerae* are encoded by the cholera toxin genes that are carried in the genome of the CTXϕ filamentous phage; thus, *V. cholerae* virulence depends on the delivery of these genes into bacterial cells via phage infection. Phages target their hosts via specific interactions with receptor proteins on the exterior of the bacteria and the receptor for CTXϕ is the intestinal colonization factor TcpA. Quite remarkably, TcpA is encoded in the VP1 PAI that is, in fact, encoded by a lysogenic phage (VPIϕ). Thus, acquisition of the cholera toxin genes by *V. cholerae* requires prior lysogenization of the bacterial cells by the VP1-encoding phage. Other examples of phage-mediated transfer of virulence factors can be found, among others, in *S. enterica*, *E. coli*, and *Streptococcus pyogenes*.

6.2 Conjugation

Conjugation, often called bacterial mating, generally requires direct contact between the donor and recipient cells and, in many cases, depends on the formation of specialized mating structures for the transfer of DNA—the *sex pilus* in Gram-negative bacteria, and an *adhesion-mediated cell–cell attachment mechanism* along with construction of a transfer apparatus in Gram-positive bacteria [130]. Conjugation is normally associated with the transfer of plasmids, generally circular, extra-chromosomal DNA molecules that self-replicate and are partitioned to daughter cells during cell division. Some plasmids, typified by the *E. coli* F plasmid, encode the complex molecular machinery required for conjugation, while others can transfer via piggy-backing

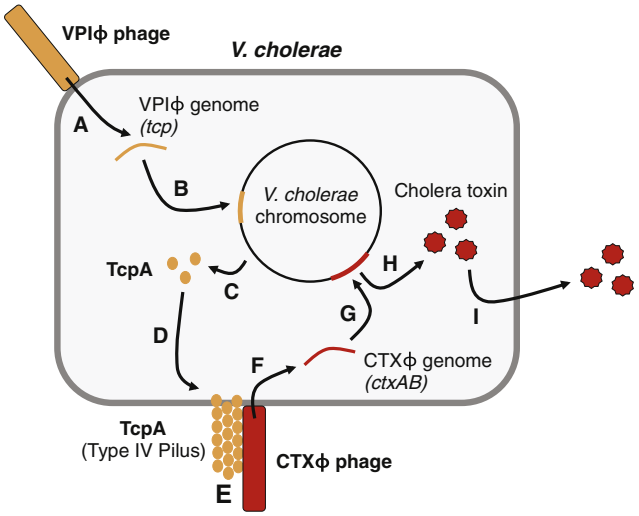

FIGURE 5.4 **Virulence acquisition of *Vibrio cholerae* via phage infection.** Virulence in *V. cholerae* is conferred by the secreted cholera toxin protein, encoded by the phage CTXϕ. Infection by CTXϕ requires previous infection and lysogeny by the VPIϕ phage and occurs via a sequential process. (A) VPIϕ injects its genome into the host cell. (B) The phage DNA integrates into the host genome leading to the stable expression of the TcpA protein from the *tcp* gene cluster (C), which forms the type IV pilus on the cell surface (D). The type IV pilus acts as the receptor for CTXϕ, which binds (E) and injects its genome into the host cell (F). The CTXϕ is integrated into the host genome (G) where it expresses the cholera toxin encoded by the *ctxA* and *ctxB* genes (H). Finally, the CtxAB dimer is secreted leading to the virulent effects on the host (I).

with other conjugative plasmids. In some cases, plasmids can integrate into the host genome resulting in stable heritability and providing an additional mechanism for the transmission of host DNA during conjugation (exemplified by the Hfr lifestyle of the F plasmid). Plasmids can encode a range of nonessential factors that can confer phenotypic variation including changes in virulence, antibiotic resistance, and adaptability to different niches. Conjugative plasmids that carry antibiotic resistance genes are an important agent of antibiotic resistance dissemination in clinical settings. This phenomenon is well illustrated by the RP1 plasmid, first identified in a clinical isolate of *P. aeruginosa* [131]. Quite remarkably, RP1 appears to be transmissible to most, if not all, Gram-negative bacteria and is a potent disseminator of antibiotic resistance, as it encodes resistance to carbenicillin (ampicillin), neomycin, kanamycin, cephaloridine, and tetracycline [132].

In contrast to plasmids, which can self-replicate, ICEs, including certain complex transposons, can also be transmitted by conjugation. These elements integrate into host chromosomes for replication, but then excise and transfer themselves from one cell to another. Insertion and excision of ICEs can cause similar chromosomal changes as insertion sequence elements (discussed earlier). The conjugative transposon Tn*5397*, originally identified in *Clostridium difficile* [133], confers tetracycline resistance and can transfer between *C. difficile* (where it has a strong insertion site preference) and *B. subtilis* (where its integration sites appear to be nonspecific) [134,135]. Tn*5397* has also been found in *Enterococcus faecalis* [136] and oral *Streptococcus* [137], suggesting that this ICE likely transfers in natural environments and could mediate horizontal transfer of antibiotic resistance.

6.3 Transformation

Transformation is a process by which naturally competent bacteria take up naked DNA from the surrounding environment [138]. The foreign DNA then typically integrates into the host chromosome either by HR, or via nonHR encoded by the foreign DNA. While the ability to take up DNA by transformation varies between different bacterial species (*competence*), transformation is a potent mechanism for the transfer of DNA between vastly divergent species. In some cases, transformation is mediated by specific recognition sequences (eg, *H. influenzae* and *N. gonorrhoeae*), but it can also be sequence independent (eg, *B. subtilis* and *Streptococcus pneumoniae*).

Natural transformation was first discovered by Frederick Griffith when he studied how *S. pneumoniae* could switch between virulent and nonvirulent strains [139]. This transformation occurred when a capsule-free nonvirulent strain took up free DNA from a heat-killed virulent strain that encoded a protective capsule. A more common function for transformation in virulence dissemination comes from the oral pathogen *Porphyromonas gingivalis* [140]. When the bacteria form biofilms on the teeth and gums, free DNA can be transferred between virulent and nonvirulent cells.

7. CONCLUSION

Bacteria live in complex environments that require rapid responses to changing conditions, as well as continued adaptation to new niches and hosts. Genome damage and resulting instability due to exposure to DNA-damaging agents, such as chemical mutagens and endogenous metabolic byproducts (eg, reactive oxygen species), is generally considered a threat to bacteria survival; however, the examples discussed here present a different side of genome instability. Bacteria have harnessed genome instability as an important element in regulating their dynamic lifestyle, by controlling gene expression in response to various stresses and stimuli. Furthermore, genome instability is an important part of the generation of genetic diversity, especially via HGT, and represents an important component of bacterial adaptation and evolution. These examples have further demonstrated the importance of genome instability in pathogenic organisms and highlighted the potential impacts on human health. Ongoing research is sure to reveal new facets of these systems, expand our basic knowledge of emerging systems (eg, bacterial MITEs) and long-studied models (eg, stress-induced mutagenesis). Almost certainly new examples of bacterial genome instability will surface as science delves deeper and deeper into the often-underappreciated complexities of bacterial genomes.

GLOSSARY

Contingency loci Simple sequence repeats often located within genes or regulatory regions that are involved in the production of surface proteins. In many cases, contingency loci facilitate responses to environmental cues through genetic rearrangements.

Integrons Mobile genetic elements that capture gene cassettes via recombination.

Phase variation Changes in protein expression, often via on–off systems controlled by genetic rearrangement. They are often involved in responding to rapidly changing environments.

SOS response A bacterial response, first characterized in *E. coli*, to DNA damage. Functionally, it is somewhat analogous to a eukaryotic cell cycle checkpoint.

LIST OF ABBREVIATIONS

bp Base pair
CF Cystic fibrosis
CRI Chronic respiratory infection
DSB Double-strand break
HGT Horizontal gene transfer
ICE Integrative conjugative element
kb Kilobase
MGE Mobile genetic element
MITE Miniature inverted-repeat transposable elements
MMR Methyl-directed mismatch repair
PAI Pathogenicity island
Pol III DNA polymerase III
Pol IV DNA polymerase IV
Pol V DNA polymerase V
polyP Inorganic polyphosphate
Ppk Polyphosphate kinase
Ppx Exopolyphosphatase
ssDNA single-stranded DNA
SSR Simple sequence repeat
$\sigma^{24/E}$ RpoE sigma factor
$\sigma^{32/H}$ RpoH sigma factor
σ^{38} RpoS sigma factor

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