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REVIEW

Recombinational Rearrangements in Bacterial Genome and Bacterial Adaptation to the Environment

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Abstract—The rearrangement of bacterial chromosomes induced by intragenomic recombination is considered. The role of stochastic and programmed genome rearrangements in bacterial adaptation to the environment and in cell differentiation is discussed.

The genome of bacteria, like the genome of other organisms, is fairly conservative. Changes in the genome are primarily due to mutations and hybridization (the latter is sometimes called the horizontal flow of genes). Recombination is a necessary step of hybridization, since foreign genes and their blocks are integrated into chromosomes through recombination. The recombination between different regions of the same chromosome, between sister chromosomes during their replication, and so on can also contribute to genome plasticity. The genome varies at a relatively high rate, providing for the natural selection of optimal genetic variants. The reversibility of some recombinational genomic rearrangements is an essential feature that makes for a prompt adaptation of bacterial populations (but not each individual) to unfavorable conditions (such as increased concentrations of toxic compounds in the medium) or for the defense of pathogenic bacteria from the immune system of host organisms. When necessary, the changed bacterial population can return to the initial state. Such system of a prompt response makes the adaptivity of bacterial populations so flexible that the adaptive response of a population is perceived as the sum of regular responses of individual cells. In some cases, however, bacterial genome in fact undergoes coordinated recombinational rearrangement in each cell of the population, presumably in response to an external signal.

The molecular mechanisms underlying recombinational variability will be considered only briefly. The relevant literature is so vast that in many cases I shall make reference to review papers and not to original articles. For the same reason, I did not dwell on the variability of actinomycetes and rhizobia and the bacterial genome rearrangements caused by the excision and insertion of phages and mobile elements.

To make the paper more readable, the description of recombinational rearrangements influencing a certain process or cellular structure begins with a short description of this process or structure.

1. MAJOR MOLECULAR MECHANISMS INVOLVED IN THE RECOMBINATIONAL REARRANGEMENT OF BACTERIAL GENOME

Recombination is an exchange of DNA elements between two chromosomes or between two segments of the same chromosome. Recombination is preceded by the conjugation (or synapsis) of these chromosomes or segments. The recombination of relatively homologous DNA segments (to be homologous, bacterial segments must contain no less than 25–30 homologous base pairs (bp)) is called homologous, scheduled, or general. Such recombination is dependent on the RecA protein. When conjugated DNA sequences are not homologous or contain only several homologous base pairs, the recombination is referred to as unscheduled. But if the conjugated sequences are unique, i.e., each of them occurs only in one or few regions of the genome, the recombination is called site-specific or specialized. The most important enzymes of the last two types of recombination are topoisomerases. The rearrangement of bacterial genomes may involve all types of recombination.

1.1. Gene Duplication and Amplification

Gene duplication is the formation of two gene copies, and gene amplification is the formation of many gene copies. Duplicated and amplified genes result from the so-called unequal crossing-over, when homologous (less frequently, nonhomologous) DNA segments located in different regions of two replicating chromosomes (typically, sister ones) recombinate. As a result, one chromosome will carry a deletion, and the other chromosome will receive a duplicated gene (Fig. 1a). A series of such events leads to gene amplification. Duplication and amplification are typical of genes neighboring direct nucleotide repeats (it is these homologous repeats that are subject to synapsis). In most cases, gene amplification depends on the RecA protein. The reverse process, when deletion sequentially removes, one by one, amplified genes, is known as deamplification. Gene duplication and amplification

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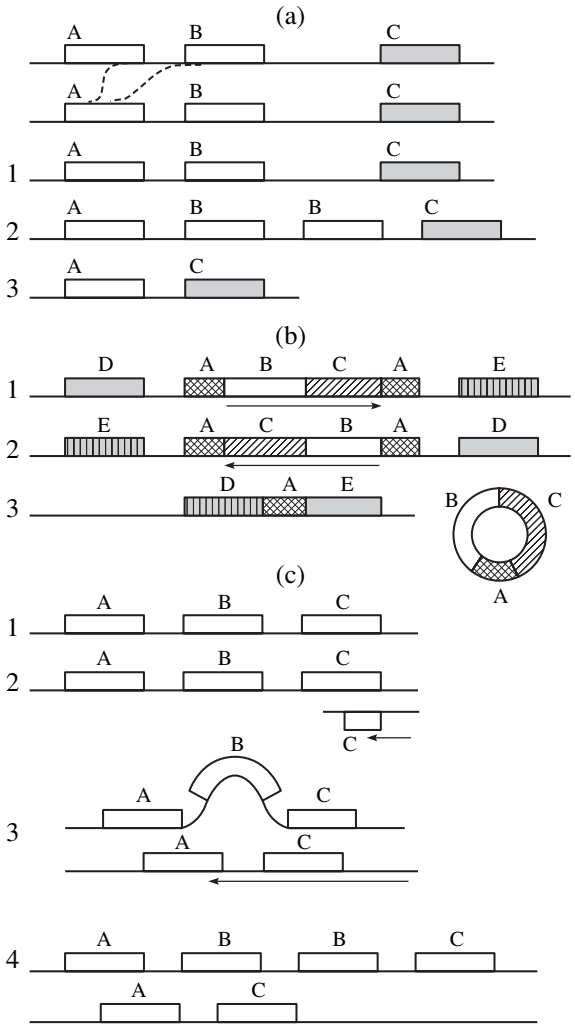

Fig. 1. (a) Homologous recombination between two sister chromosomes. Homologous DNA segments A and B are located in different regions of the chromosome. Nonhomologous segment C is flanking. Recombination is shown by dashed lines. (1) The recombination between segments A does not alter the chromosome structure. (2) The recombination between segments A and B (unequal crossing-over) results in the formation of a chromosome with the duplicated segment B. (3) The recombination between segments A and B (unequal crossing-over) results in the formation of a chromosome with the deleted segment B. (b) Formation of inversions and excisions by unscheduled recombination. (1) The original structure represents the central segment BC of the chromosome with boundary repeats A. Segments D and E are flanking. (2) The inversion product represents the inverted central segment CB. (3) The excision product represents the central segment excised in the form of a circular structure. The chromosome in this region has a deletion. The flanking segments D and E become closer, with one of the repeats A between them. (c) Replication of DNA by the slipped-strand mispairing mechanism. (1) The original DNA segments A, B, and C. (2) The onset of replication. (3) The slipping of segment B during replication. (4) The product of the slipped-strand base mispairing replication: the upper DNA strand carries the duplicated segment B and the lower DNA strand carries a deletion of segment B.

are the simplest consequences of unequal crossing-over [1–4].

1.2. DNA Inversion and Excision

Inversion is the rotation of a chromosomal segment through an angle of 180° within the same location, which is due to different recombination mechanisms. Site-specific recombination is dependent on topoisomerases (also called invertases or recombinases), which act on direct repeats with two unique boundary sequences, 5 to 20 bp in size. Invertases, each of which is strictly specific for its repeat, bind to the repeats of replicating chromosomes, break all four DNA strands of these chromosomes (without the involvement of any nucleases), and rotate them through 180° (Fig. 1b). Invertases are aided by specific proteins, such as FIS (factor of inversion stimulation), which likely bend DNA strands during inversion. Of interest is the fact that the structural genes of invertases are often located near their target repeats. Site-specific recombinases also promote the excision of DNA segments and thereby the formation of deletions [5].

1.3. Slipped-Strand Base Mispairing

As in the case of recombination, slipped-strand base mispairing causes gene duplication and deletion. According to one of the models simulating this phenomenon, DNA polymerase slips along a region of a replicating matrix DNA strand, resulting in a blank in the newly synthesized DNA strand. Accordingly, the matrix strand forms a mispaired loop, which, in turn, can serve as an additional matrix for the replication of the sister chromosome. This may result in duplicating the corresponding DNA segment. After the replication is over, one of the new DNA strands will carry a deletion, whereas the other will carry a duplication (Fig. 1c). Slipped-strand base mispairing is often observed during the replication of a DNA segment with multiple repeats of the same base pair (the so-called homopolymeric tract). As in unequal crossing-over, the RecA protein is not involved in slipped-strand base mispairing [4, 6].

2. INCREASED GENE DOSAGE

The notion of gene dosage was first introduced to define ploidy or the number of homologous chromosomes. For instance, *Drosophila* males have one X-chromosome, while *Drosophila* females have two X-chromosomes, which results in a twice as much dosage of certain genes in *Drosophila* females. Now the notion of gene dosage is also used with reference to gene duplication and amplification.

The adaptation of bacterial populations to the environment may occur through increase in the amount of certain proteins valuable for fitness. This can be exemplified by the amplification of the lactose operon genes

in *Escherichia coli* grown in media with limiting concentrations of lactose as the sole source of carbon and energy. The cells of this bacterium with the amplified β-galactosidase gene grow faster and gradually accumulate in the cultivation medium [7–9].

Amplification can augment gene dosage by 40–200 times. The size of amplified DNA segments may reach 30 kbp. Sonti and Roth described the role of gene amplification in the adaptation of *Salmonella typhimurium* to growth on limiting carbon sources (arabinose and sorbitol) [10]. Other examples of the effect of gene amplification on the amount of protein products can be found in the review of Anderson and Roth [1].

Gene amplification can promote the selection of organisms resistant to antibiotics and toxic compounds. Such resistance is due to chromosomal mutations or, more frequently, to the penetration of plasmids carrying resistance gene into cells and the amplification of these genes. Such plasmids can be integrated into chromosomes and be amplified in the integrated state. For instance, when integrated into the *Bacillus subtilis* chromosome, the staphylococcal plasmid p*C*194 with the chloramphenicol resistance gene was amplified 15-fold by subculturing the bacterium in media with the gradually increasing concentration of the antibiotic [11]. Similarly, the chloramphenicol and erythromycin resistance plasmid pGG10 was found to be amplified when integrated into the *B. subtilis* chromosome [12]. More information on the amplification of bacterial and plasmid resistance genes can be found in the reviews of Vel'kov [2], Petes and Hill [3], and Khasanov *et al.* [13].

Gene amplification is a random process occurring at a rate of 10^{-2} to -10^{-5} per cell. The gene amplification rate does not depend on the presence of selective agents in the medium. If a population of cells with an amplified gene is cultivated in the absence of any selective agent, cells with a diminished number of the amplified gene begin to accumulate in the medium (such cells are always generated in the population, but they do not accumulate in the presence of the respective selective agent). Like gene amplification, gene deamplification is also cased by recombinational mechanisms.

Gene amplification can be stable and very intense. For instance, the amplification of the glycyl-tRNA synthetase gene in *S. typhimurium* gave rise to a redundant chromosome fragment, whose dose reached 25% of the entire genome of this bacterium [14].

As was mentioned above, the unequal crossing-over between homologous chromosomal regions can lead to scheduled gene duplication. Such regions, which may be large or small and be closely or widely spaced, are numerous on bacterial chromosomes. Some of them, such as the 4- to 5-kbp *rhs* repeats in the *E. coli* genome, seem to be specially destined for gene duplication [15]. In any event, other functions of these repeats are not as yet revealed. Some ribosomal and tRNA genes, IS elements [16], and small homologous DNA segments can also be duplicated. As for unscheduled gene duplication, it is due to RecA-independent nonhomologous recombination (see above).

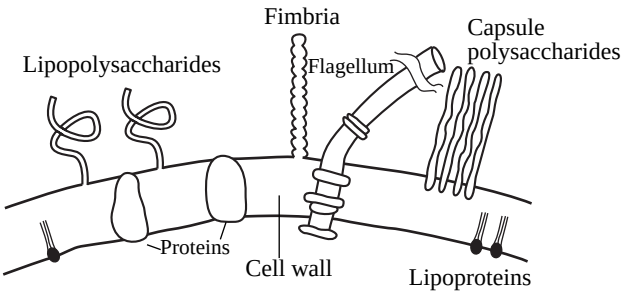

Fig. 2. The cell-wall structures of gram-negative bacteria (a modified drawing after Moxon *et al.* [32]).

Because of methodological difficulties, few natural amplificants (i.e., strains carrying adaptive gene amplifications) are known. One of these, *Vibrio cholerae* El Tor is only slightly virulent and toxic. However, the introduction of this vibrio into the rabbit intestines allowed the isolation of several hypertoxic clones, whose genetic analysis showed that they carried the amplified toxin operon. The hypertoxicity of these clones offered them advantage in the colonization of the rabbit intestines. The amplified DNA segment had homologous boundary repeats and required the *recA* gene for amplification [17, 18].

Gene duplication and amplification in bacteria and, probably, in all living organisms serve not only to increase gene dosage and to enhance the production of the respective protein but also may play an important evolutionary role. The duplication of genes is believed to precede their divergence. One of the duplicated genes no longer undergoes selective pressure (as the other gene does the work of two) and, due to accumulated mutations, may transform to a pseudogene and then to a gene with a different function [19, 20]. The evolution of genes and proteins is, however, beyond the scope of the present review.

3. VARIABILITY OF THE SURFACE STRUCTURES OF PATHOGENIC BACTERIA

Pathogenic bacteria invading a host organism are attacked by many immune factors present in this organism. In turn, pathogenic bacteria possess virulence factors, which allow them to counteract the immune attack of the host organism and to colonize it. The key part in bacterial virulence is played by the variability of various cell-surface and cell-wall structures and components, such as flagella, pili, capsules, cell-surface proteins, lipoproteins, and liposaccharides (Fig. 2). The variability of these structures and components is mainly due to the rearrangement of the respective segments of the bacterial genome. On the other hand,

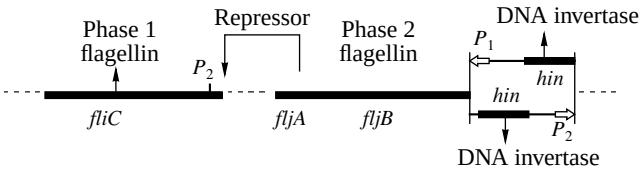

Fig. 3. A scheme illustrating the phase variation of flagellins in salmonella (a modified scheme after Khesin [19]). Designations: *fliC* and *fljB*, the phase 1 and phase 2 flagellin genes, respectively; *fljA*, gene encoding the *fljC* repressor; *P1*, the common promoter of the *fljB* and *fljA* genes; *P2*, the *fliC* gene promoter; and *hin*, the Hin invertase gene.

similar mechanisms provide for the variability of immunoglobulins, which are produced by B lymphocytes and represent one of the major defense systems of host organisms [21].

3.1. Flagella

Flagella are the complex organelles of bacteria responsible for their motility. The structure and function of *E. coli* flagella are controlled by no less than 40 genes. One enteric bacterial cell has 5–6 peritrichous flagella. The flagellum consists of a filament, about 10 μm in length and 20 nm in diameter, a basal body, which serves as a motor rotating the filament, and a hook (a curved extension of the filament penetrating the cell wall and connecting the filament with the basal body). Flagella are made of molecules of a specific protein, flagellin [22]. This protein is one of the primary surface antigens of flagellated bacteria.

As far back as 1922, experiments on the serotyping of the causative agents of salmonelloses showed that *Salmonella* populations always contain two types (or phases) of cells differing in the antigenic specificity of flagellins (the so-called phase variability). The antigenic specificity of cells reversibly varied at a rate of 10^{-3} to 10^{-5} per generation.

Genetic studies initiated in the mid-1950s showed that the phase 1 and phase 2 flagellins of salmonella are encoded by the *fliC* and *fljB* genes (formerly, *H1* and *H2* genes). The flagella of salmonella are composed of either phase 1 flagellin or phase 2 flagellin molecules. The *fljA* gene, which is under the control of the same promoter as the *fljB* gene, codes for the synthesis of the *fliC* gene repressor. When the *fljA* and *fljB* genes are active, only phase 2 flagellin is synthesized, since the *fliC* gene is suppressed.

At the left of the *fljA* and *fljB* genes, overlapping their common promoter, there is a 995-bp DNA segment containing the recombinase *hin* gene and its promoter (Fig. 3). This sequence has two 14-bp inverse boundary repeats. In the process of unscheduled recombination, the *Hin* invertase rotates this fragment through 180° at a phase variation frequency. In this case, the common promoter of the *fljA* and *fljB* genes is also inverted and, therefore, is switched off. As a result, the syntheses of phase 2 flagellin and the *fliC* gene repressor are suspended, while the synthesis of phase 1 flagellin is activated [19, 22–24] (Fig. 3). The biological significance of phase variation in *S. typhimurium* is most likely to provide defense against the antibodies of the host organism.

3.2. Fimbriae

Along with flagella, bacterial cells may contain other hair-like extracellular appendages, which are called either fimbriae or pili. Certain pili (called sex pili) allow one bacterium to recognize and adhere to another in a process of sexual mating called conjugation. Fimbriae are shorter and thinner than flagella and consist only of protein subunits. Fimbriae are more numerous than flagella, their number ranging from 20 to several hundreds per cell. Some bacteria, like *E. coli*, have several types of fimbriae.

The biological role of fimbriae is to allow bacterial cells to attach to solid surfaces, including the surface of cells of a host organism. In view of this, the fimbriae of pathogenic bacteria are one of the virulence factors. Certain fimbriae are responsible for the twitching motility of cells [25, 26].

3.2.1. Gonococcal pili. Gonococcal cells, which are diplococci, contain several tens of pili made up of polymerized pilin molecules. These pili, together with the Opa proteins (see below), play the key role in the attachment of gonococci to the epithelium of the urogenital tract and to leukocytes in gonorrheal patients (up to 200 diplococci may be attached to one leukocyte). Experiments to study the growth of gonococci on agar media showed that the bacteria freshly isolated from such patients produced typical small colonies on agar [27]. However, some colonies were larger than the others and contained cells lacking pili. Analysis showed that these cells are not pathogenic. The subculturing of cells from large colonies gave rise to small colonies containing gonococcal cells with pili. By analogy with salmonella, such transitions between cells either furnished or not with pili were called phase variations. The antigenic properties of gonococci are fairly variable, largely due to changes in gonococcal pilin. The antigenic variability of gonococci allows them to live and reproduce for a long time in the urogenital tract and other organs, counteracting the attack of antibodies. This is also the reason for the absence of acquired immunity to gonorrhea. The antigenic variability of pilin and the antigenic and morphological variability of gonococcal cells (the presence or the absence of pili) were described in detail elsewhere [28–33].

The mechanism of phase variations in gonococci turned out to be different from that in the case of the flagellin of salmonella. The synthesis of gonococcal pilin is controlled by 12 tandem genes separated by DNA segments containing short repeats [34, 35]. Only one of these genes, *pilE1*, is capable of normal expres-

sion, whereas the other pilin genes (called silent genes) are not normally expressed, since they lack promotes and have some other defects. These genes are located in the *pilS* DNA segment and recombinate with the *pilE1* gene at a high rate, due to which this gene undergoes mutations (primarily, frame-shift mutations). As a result, gonococcal recombinants either completely lack pilin and pili or may have pilin and no pili. Furthermore, gonococci may be furnished with morphologically normal but antigenically altered pili [29, 31, 33, 36].

All these changes are due to homologous recombination. The deletion of the *recA* gene reduces the frequency of phase variations by several hundred times [37, 39]. The deletion of the *recD* gene, which is also involved in homologous recombination, reduces the phase variation frequency by 12 times [38].

Genetic transformation considerably contributes to the variation of pilin in gonococci. Unlike cells lacking pili, the cells that have pili can absorb foreign DNA in any growth phase [40]. The spontaneous transformation of gonococci occurs at a high rate due to DNA releasing from autolyzed cells. The pilin genes, as well as other DNA segments, are continually integrated into the chromosomes of recipient cells. This type of gonococcal recombination depends on the RecA protein [41]. The addition of DNase to the cultivation medium diminishes, albeit not to zero, the frequency of phase variation, evidently due to the degradation of extracellular DNA. Gonococci spontaneously transform in cultures and, likely, in infected humans. The existence of the phase variation of gonococcal pili in strains unable to absorb extracellular DNA suggests that some mechanisms, other than spontaneous transformation, are involved in the phase variability of gonococci [42].

The combined action of all these recombinational processes causes a continuous conversion of the *pilE1* gene and determines the tremendous phase and antigenic variability of gonococci. This makes very difficult the creation of antigonococcal vaccine [36]. The conversion of the pilin region of the gonococcal genome is schematically depicted in Fig. 4.

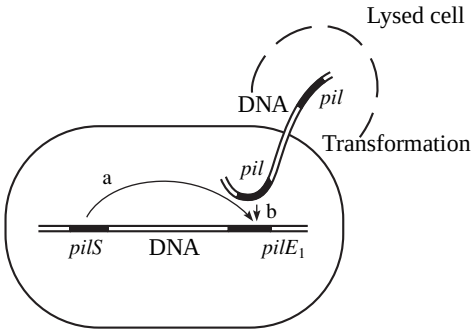

Fig. 4. A scheme illustrating two types of pilin formation in gonococci (a modified scheme after Saunders [29]): (a) intragenomic recombination and (b) intragenomic recombination due to spontaneous transformation. Designations: *pilS*, silent pilin gene and *pilE1*, active pilin gene.

3.2.2. Fimbria of hemophilic bacteria. Hemophilic bacteria, the causative agents of meningitis in humans, also have fimbria, which promote the attachment of these bacteria to epithelial cells and facilitate nasopharyngeal colonization. The strains of hemophilic bacteria isolated from diseased humans always have fimbriae. However, the cultivation of these strains on agar media often gives rise to bacterial variants lacking fimbriae. In other words, like gonococci, hemophilic bacteria also undergo reversible phase variation [43].

The formation of fimbriae in hemophilic bacteria is controlled by the chromosomal region containing two genes, *bifA* (encodes polypeptide subunits) and *bifB* (encodes a protein similar to periplasmic ·haperones). The formation of fully functional fimbriae requires both genes, which are arranged in such a manner that they are transcribed in the opposite directions and their promoters overlap (i.e., their coding regions lie outward, while their overlapping promoters lie inward of the respective DNA segment). The alternate switch-on and switch-off of fimbriae synthesis occur at a high rate due to changes in the promoter regions where the TA repeats are located and where RNA polymerase is attached to the promoter (this region lies between the –35 and –10 nucleotides). The optimal number of the TA repeats for the attachment to be efficient is equal to 10. If this number is lower or greater by 1–2 repeats, the transcription of this region is impaired. Actually, because of slipped-strand base mispairing, the number of the TA repeats continually changes. This is the reason why the synthesis of fimbriae is alternatively switched on and switched off [32, 44].

3.2.3. *E. coli* fimbriae. *E. coli* cells have fimbriae of different types. Type 1 fimbriae are typical of virulent strains of this bacterium [45]. These fimbriae undergo phase variations at an abnormally high rate. For instance, over a time span equal to one generation, up to 70% of cells in a population may appear to have no fimbriae at all. The reverse process goes at a rate lower by a factor of about 700 [46].

The formation of type 1 fimbriae is controlled by the *fimA* gene, which lies to the left of the *fimB* and *fimE* genes. The *fimA* gene promoter is located between the *fimE* and *fimA* genes, in a 324-bp DNA segment with 9-bp inverted boundary repeats.

The *fimB* and *fimE* genes encode two invertases, which are responsible for the unscheduled recombination of the inverted repeats of this region of the chromosome. As a result, the 314-bp segment is inverted together with the promoter, switching off the synthesis of fimbrial proteins. The switch-off can be implemented by either of the two invertases, while the switch-on (i.e., the back inversion of the DNA segment together with the promoter) can be implemented by only the *fimB*-encoded invertase [26, 47–49]. The inversion requires the integration host factor (IHF), a DNA-binding protein that stimulates unscheduled

recombination in different regions of the chromosome. This factor is also involved in interactions between the *E. coli* chromosome and phage lambda [50].

3.2.4. *Proteus mirabilis* fimbriae and *Moraxella bovis* pili. The bacterium *P. mirabilis* has fimbriae of four types. The virulent freshly isolated strains causing pyelonephrosis and stone formation in human kidneys and urinary bladder have the MR/P fimbriae. Most *P. mirabilis* cells cultivated on agar media have no fimbriae. Under certain cultivation conditions, however, many cells show the presence of fimbriae, presumably due to selective pressure or some increase in the frequency of phase variation. After 7 days of the experimental infection of laboratory mice with *P. mirabilis*, all isolated strains possess fimbriae. The evident relationship between the pathogenicity and fimbriae of *P. mirabilis* can be explained by the role of the fimbriae in the attachment of this bacterium to the urinary tract epithelium. It can be suggested that unfimbriated cells either are washed away from the epithelium by flowing urine or are otherwise inactivated [51].

The molecular mechanism underlying the phase variation phenomenon in *P. mirabilis* is very similar to that of *E. coli*. The 252-bp DNA segment with two 21-bp inverted boundary sequences is responsible for the synthesis of the main protein component of fimbriae. Further to the left, the *mrlP* gene is located, which most probably encodes recombinase resembling the FimB and FimE recombinases of *E. coli.* The function of this recombinase is to perform the reversible inversion of the 252-bp segment with the accompanying switch-on and switch-off of the *mrpA* gene. When the lactose operon genes lacking their native promoter are built-in on the right of the *mrpA* gene, the colonies grown on the respective media with a chromogenic substrate vary not only in the ability to produce fimbriae but also in the ability to utilize this substrate [52].

Let us now briefly consider the recombinational variability of the pili of *Moraxella bovis*, the causative agent of conjunctivitis in cattle. The pili of this bacterium are obviously needed for its attachment to corneal cells: the *M. bovis* strains lacking pili are not pathogenic. The pili affect the colonial morphology and the competence of *M. bovis* cells. The phase variability of this bacterium is manifested in the alternate synthesis of two pilin types, α-pilin and β-pilin with molecular masses of about 18 and 16 kDa, respectively. The phase variability is determined by the inversion of a 2-kbp DNA segment containing the genes of both pilins [53].

3.3. Cell-Wall Proteins, Lipoproteins, and Liposaccharides

Bacterial cell-wall proteins, lipoproteins, and liposaccharides perform not only structural but also other functions, such as the attachment of pathogenic bacteria to host cells and the protection of the former against the bactericidal action of the host blood serum. The high variability of cell-surface proteins, lipoproteins, and liposaccharides is essential in maintaining the immunity of host organisms at a sufficient level and has, therefore, become the subject of particular interest from clinical microbiologists and geneticists.

3.3.1. The Opa proteins of *Neisseria*. Along with pilin and pili, the Opa proteins (or PII proteins) of gonococci play an important part in their virulence and ability to attach to the host organism epithelium. The phase variation of the Opa proteins, which occurs at a rate of 10^{-3} to 10^{-4} per cell generation, affects the color, morphology, and opacity of gonococcal colonies (it should be noted that the name Opa is derived from the term *opacity*) and can induce some other antigenic alterations not manifested in colonial morphology. The *opa* genes of gonococci (at least eleven) are spread over the chromosome. The transcription of these genes is constitutive; therefore, the phase variation of the Opa proteins is realized at the translational level [54].

Each of the *opa* genes contains a number (up to 27) of direct 5'-CTCTT-3' repeats located, in particular, in the DNA region encoding a hydrophobic segment of the leader peptide of the respective protein. The deletion or insertion of additional repeats causes frameshift mutations. This process is independent of the RecA protein and is likely due to slipped-strand base mispairing [55]. Murphy *et al.* constructed a plasmid in which one of the *opa* genes was located with respect to the alkaline phosphatase gene in such a manner that the expression of the latter gene depended on the number of the repeats in this *opa* gene, i.e., on its phase variability. The introduction of this plasmid into *E. coli* induced the phase variation of this bacterium, which itself could easily be observed due to the different color of colonies with the alternate expression of the phosphatase gene [55].

Similar phase variations in the *opr* genes (analogues of the *opa* genes) were observed in other *Neisseria* species and in meningococci [29].

3.3.2. The lipoproteins of *Borrelia hermsii* and *Mycoplasma hyorhinis*. Different clones of the spirochete *B. hermsii* carry one of the 26 serologically different lipoproteins. At least two variants of the variable major protein (VMP), variants 7 and 21, are encoded by genes located on one of the linear plasmids of this bacterium. Normally, these genes are silent. However, after recombinating the carrying linear plasmid with another linear plasmid, the *vmp* gene variant 7 begins to express. But if this gene is deleted from the carrying plasmid due to recombinational rearrangement [57], the *vmp* gene variant 21 is expressed. The alternate expression of these genes is triggered at a rate of 10^{-4}.

Similar phase variations of lipoproteins are described for *M. hyorhinis*, the causative agent of the chronic arthritis of pigs [58].

3.3.3. The liposaccharides of hemophilic bacteria and gonococci. The liposaccharide layer of hemophilic bacteria isolated from the nasopharynx is believed to

protect them against the humoral immunity (an immunity due to the combined action of antibodies, complement, and C-reactive protein) of host organisms [60]. The liposaccharide molecule consists of a lipid moiety anchored in the cell wall and a protruding carbohydrate moiety [60]. The antigenic properties of the liposaccharide varies at a rate of 10^{-2} to 10^{-3} or higher. Phase variations are related to the expression of the *lic2* gene encoding galactoside transferase, which is needed for the synthesis of digalactoside. The 5'-terminus of the *lic2* gene contains about 16 tandem CAAT repeats (their number changes slightly due to slipped-strain base mispairing) [61]. The repeats are located upstream the initiating codon [31, 32, 62]. The phase variation of phosphocholine is related to changes in the translation of the *lic1* gene [63].

Gonococcal liposaccharides also undergo reversible antigenic variations, whose underlying mechanism is unknown except that it is independent of the RecA protein [42].

3.4. Capsules

Capsule represents a thick surface layer of cells, usually visible under the light microscope, which lies outside the cell wall, if it is present. The capsule can be reversibly removed from a cell without considerable damage to its viability. The capsule has a polysaccharide nature.

3.4.1. The capsules of hemophilic bacteria. Hemophilic bacteria produce polysaccharide capsules consisting of ribose and ribitol-5'-phosphate. The capsules make hemophilic bacteria resistant to phagocytosis. About 95% of *Haemophilus influenzae* type B strains freshly isolated from meningitic and other patients are encapsulated. However, any colony grown on agar contains from 0.1 to 0.5% of decapsulated cells, which are avirulent and produce small slow-growing colonies when subcultured. The high rate of decapsulation suggests that it is caused by phase variation [30, 32, 64].

Capsule formation is controlled by the *cap* gene. The *H. influenzae* type B chromosome usually carries two *cap* genes connected by a 1.3-kbp bridge. Each of the *cap* genes has a flanking IS-1016 sequence [64, 65], indicating that this DNA segment is a complex transposon. The bridge carries a small gene, *bexA*, whose function is to export polysaccharides to the cell surface. Upon phase variation, this gene and one of the *cap* genes are deleted, as a result of which the polysaccharides synthesized with the involvement of the other *cap* gene cannot reach the cell surface, and cells remain noncapsulated. Gene deletions are due to the homologous recombination of the *cap* genes arranged in the form of a direct repeat [61, 66]. The recombination of the *cap* genes may lead to their amplification by a factor of up to 5 [32].

4. REGULATION OF SOME STAGES OF SPORULATION IN BACILLI

Sporulation is a complex adaptive multistep process, taking up to 8 h in *Bacillus subtilis*. Sporulation includes, in particular, the duplication of the nucleoid, one of which will be transferred to the spore. The formation and germination of spores is controlled by many genes (more than 150 in *B. subtilis*). After the segregation of duplicated nucleoids, their genes continue to function for about 4 h in two cell compartments, the mother compartment and the so-called prespore (or forespore). In essence, these two compartments represent a two-cell organism bounded by a common cell wall. The functioning of sporulation genes is regulated by triggering sigma factors, so that some genes are active in the mother compartment and are suppressed in the prespore and vice versa. It should be noted that actually the sigma factors regulate entire sporulation operons and even groups of such operons.

The triggering mechanism of sporulation regulation involves intrachromosomal recombination, which leads to the excision of certain DNA segments. It is this process that initiates the formation of one of the sigma factors, sigma K, in the mother compartment 3 h after the beginning of sporulation [67]. The sigma K factor allows the transcription of silent genes necessary for spore maturation to set in.

In vegetative *B. subtilis* cells, the *sigK* gene consists of two unequal parts separated by a 48-kbp DNA segment (the so-called skin element, which will be described below in more detail). The larger part, *spoIVCB*, codes for the protein domain corresponding to the NH_2-terminal portion of the sigma factor, whereas the smaller part, *spoIIIC*, codes for the COOH-terminal portion of the sigma factor [68, 69]. The joint region of these two portions of the sigma factor corresponds to the –10 promoter region. In vegetative cells, the sigma factor is not functional because of the segment separating the two gene parts. However, 3 h after the onset of sporulation, the unscheduled recombination of the mother chromosome will lead to the excision of the separating segment and, hence, to the synthesis of the fully functional sigma factor. It should be emphasized that the altered mother chromosome will die 4 h afterwards together with the entire mother compartment, whereas the unchanged prespore chromosome will continue to exist.

As for the skin element (this name is derived from the term *sigK intervening*), it is most likely to be an uninducible prophage genome integrated into the bacillar chromosome [70, 71]. The skin element contains the functional *ars* operon with four reading frames, which controls cell resistance to arsenic and antimony. It remains unknown how this operon has appear in the bacillar chromosome, as it is homologous to the counterpart operons of staphylococci and *E. coli* [72]. The skin element is not vital to *B. subtilis*: the mutant of this bacterium deficient in the skin element was found to be

fairly viable, although it was asporogenous and sensitive to arsenic [72]. The transcription of the *sigK* gene in this mutant was not studied.

The skin element is excised from the bacillar chromosome by one of the site-specific recombinases, which is encoded by the *spoIVCA* gene located within the skin element. The recombination underlying this excision takes place between the two boundary ATTGA repeats. The excised skin element has a circular structure and can easily be seen on electrophoretograms [67]. The factor that switches on the recombinase gene at a proper time is unknown. Some role in this process is presumably played by environmental conditions.

5. REGULATION OF CYST FORMATION IN CYANOBACTERIA

Cyanobacteria are photosynthesizing microorganisms incapable of fixing molecular nitrogen under normal conditions. Some species of cyanobacteria form multicellular filaments. Under anoxic conditions and absence of nitrogen sources in the medium, approximately every tenth cell of the cyanobacterial filament converts to the so-called heterocyst. Heterocysts have several envelopes, due to which they are resistant to lysozyme and other factors. They are unable to divide, and their DNA is not replicated. Heterocysts die some time after they have been formed. The metabolism of heterocysts greatly differs from that of parent cells, since many genes necessary for photosynthesis are suppressed, while the *nif* genes (the name is derived from the term nitrogen fixation), coding for the polypeptides of the nitrogenase complex involved in nitrogen fixation, are induced. The amount of the glutamine formed by one heterocyst is sufficient to feed about 10 neighboring vegetative cells (5 cells on each side of this heterocyst in the cyanobacterial filament). In turn, vegetative cells continue to fix carbon dioxide and transfer synthesized carbohydrates to heterocysts.

A complete transformation of vegetative cells to heterocysts takes about 24 h. Eighteen hours after the onset of the transformation, the 11-kbp fragment of the heterocyst chromosome between the *nifK* and *nifD* genes, including a portion of the *nifD* gene, is excised in the form of circular DNA. The resultant modified *nifD* gene codes for a polypeptide containing 497 amino acid residues, which represents the α-subunit of nitrogenase. The *nifK* gene comes under the control of the *nifH* gene promoter, so that three genes, *nifK*, *nifD*, and *nifH*, form a functional operon. When induced, this operon makes heterocysts capable of fixing molecular nitrogen, which itself is their major function [74].

The DNA rings excised (called excisomes) are not degraded and can be detected in the heterocysts by electrophoretic methods. The functional role of excisomes is unknown. Their excision is due to recombination between the direct flanking 11-kbp repeats of the DNA segment to be excised and involves one of the site-specific recombinases, encoded by the *nifA* gene. This gene is adjacent to the left repeat and appears in the excisome after its excision has occurred [75, 76]. All this closely resembles the excision mechanism of the skin element (see above).

Another fragment of the heterocyst chromosome, containing 55 kbp, is excised under the same conditions as indicated above (anoxic environment and the absence of nitrogen sources), as well as under microoxic conditions. The segment to be excised lies at the flank of the DNA segment containing the *nif* genes, within the *fdxN* gene encoding a ferredoxin-like protein. This segment is excised with the involvement of another recombinase, *xisF*, and other flanking repeats. It was found that this chromosomal segment is transcribed neither before nor after excision; its function in vegetative cells remains unknown. The excision restores the integrity of the *fdxN* gene necessary for nitrogen fixation. Both segments, 11-kbp and 55-kbp, are excised independently [77–81].

6. CONCLUSION

The recombinational rearrangements of bacterial genome can be divided into two groups. The most common, stochastic, mutations occur at a higher rate than random point mutations. These mutations are usually reversible and profitable to bacterial populations. As Haselcorn wrote [79], “Cells with gene rearrangements are like Boy Scouts, who are always in a ready-to-act state. Even if the event to which they are prepared for has not come, this makes no problems, for similar spontaneous gene rearrangements will occur in the next generation, even though at a low frequency.” It should be noted that Haselcorn is not quite right when he speaks about the low frequency of stochastic gene rearrangements. It is known that gene duplication and amplification can occur at a frequency reaching several percent per generation. This leads to the ideas of the bacterial genome instability or plasticity [4, 13, 19], which, at first sight, disagree with the doubtless genome conservativeness [4].

While being not so numerous as stochastic gene rearrangements, programmed gene rearrangements are of particular research interest. Such gene rearrangements, which have presently been described only for bacilli and cyanobacteria, can occur almost in all cells of a population. They are involved in the regulation of some developmental stages of bacteria, such as the formation of spores and heterocysts, and take place in response to changes in environmental conditions. Were cells with such gene rearrangements able to remain viable, give offspring, and then return to the original phenotype, the Lamarckian ideas of variability and heredity would sound more realistic. However, heterocysts and the mother compartment of sporulating bacilli are not viable and die off within a short time span. The excision of two DNA segments in heterocysts and the skin element in sporulating bacilli is unlikely to be the death cause (as was mentioned above, bacilli lacking

the skin element can live and even reproduce). Rather, we deal with the prototype of cell division into somatic (mortal) cells and germ-line reproductive (potentially immortal) cells, as in eukaryotes. One of the types of gene rearrangements in somatic eukaryotic cells is chromatin diminution (DNA elimination), which may reach 94% of the genome of reproductive cells [82]. The extent of gene rearrangement processes in prokaryotic bacteria is considerably lower; however, their goal is more distinct: to provide for the functioning of the silent genes of the mother compartment, which itself is necessary for spore maturation. Once this goal has been achieved, the mother compartment becomes unnecessary and is destroyed. The mechanism responsible for the destruction of the mother compartment remains unknown.

Among many relevant publications that were not cited in the present review, those of Borst and Greaves [83], Plasterk [84], Rainey and Moxon [85], and Henderson *et al.* [86] should be mentioned.

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