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Milestones of Studying the Mechanisms of the Genetic Bacterial Transformation on the Model of *Streptococcus*

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Abstract—The chronology for the main stages of studying bacterial genetic transformation is presented. Streptococci (pneumococci in particular) were used as the model organisms. Recent data on the mechanisms of releasing DNA from bacterial cells and the role of natural transformation in the horizontal transfer of genes are considered.

Keywords: pneumococci, DNA, stages of natural transformation, horizontal gene transfer
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INTRODUCTION

Genetic transformation in bacteria plays an essential role in genetics and biology, primarily for presenting the first undeniable evidence of DNA as a carrier of heredity. In 2015, it was 71 years since the first manuscript on this evidence was published and 87 years since the first publication on the phenomenon of transformation in pneumococci was released. Some of these studies later segregated into an independent field that studies competence, a state during the stage of bacterial growth when many bacterial species are able to uptake DNA and integrate it into their own genome. A number of bacterial genes regulate competence, which is a part of the “quorum sensing” phenomenon, the interactions between cells in growing populations of microorganisms. Transformation was also discovered to play a part in the DNA transfer under natural conditions of bacterial growth; e.g., inside the human nasopharynx. Thus, the transformation, during which DNA is taken up by “the cell itself,” is called natural transformation. Later, a number of methods for the “unnatural” introduction of DNA into the cells (called artificial transformation, in contrast with the natural one; e.g., electroporation) was developed. Plasmid transformation as a method of introducing vectors with an integrated region of foreign DNA in a cell for subsequent cloning is of great practical significance.

Studies on this subject were performed at such a high rate that, since 1972, biennial European Symposiums on Bacterial Transformation were held in various European countries. There were a total of 13 symposiums (the last one in 1999).

The goal of this review is to retrace the general trend and stages of research on natural transformation in almost ninety years. To this day, transformation has been discovered in more than eighty bacterial species in different genera, with every one of them having unique features. This review, however, will focus on the studies performed on pneumococci and certain other species of streptococci. These particular bacteria served as a model and a paradigm for later research on other microorganisms. Only fundamental studies, reviews, and memoir literature will be cited in the present review.

The author of the present review studied bacterial transformation (mainly using *Bacillus subtilis* as a model) and microorganism transformation related to the field of genetics since 1961 (first, at the laboratory of R.B. Khesin); participated in most of the European Symposiums on Bacterial Transformation; published a book on this subject (Prozorov, 1988); and witnessed main stages of evolution of this field of bacterial genetics for the last fifty years.

DISCOVERY OF GENETIC TRANSFORMATION PHENOMENON AND RESEARCH ON THE ROLE OF DNA IN THIS PROCESS

It is known that English microbiologist Frederick Griffith described the transformation phenomenon in pneumococci in 1928. Pneumococcus *Diplococcus pneumoniae*, later renamed *Streptococcus pneumoniae*, is a species included within the wide genus *Streptococcus*, which induces various diseases, primarily lobar pneumonia. These bacteria possess a polysaccharide capsule. Encapsulated cells give raise to relatively large smooth S colonies, while the cells derived from a capsule (due to mutations, as was found later) produced small and rough R colonies. The capsule specifically

protects bacteria from phagocytosis, i.e., is one of the virulence factors. Pneumococci possess various serotypes that are distinguished by the composition of the capsule's polysaccharides. In the first decades of the last century, before the discovery of the sulfamide drugs and antibiotics, lobar pneumonia could be effectively cured only by injections of large volumes of type-specific serum. Because of that, serotherapy required knowledge of the serotype of the pneumococci that caused the disease in a studied patient. Griffith, in one of the experiments in his research on the mechanisms of virulence, injected mice with live bacterial cells without a capsule (thus, avirulent), which were initially serotype I, and encapsulated serotype II cells killed by heating to 60°C. The mice died, but the bacteria inoculated from their organs were encapsulated serotype II, not serotype I bacteria. Griffith called this conversion of serotypes transformation (Griffith, 1928). German researchers at the Robert Koch Institute (Berlin) immediately replicated the results of these experiments (Neifeld and Levintal, 1928). Confirmation of Griffith's results was also included in later publications (Reimann, 1929; Baumhenn, 1932). Since then, Griffith himself never published other studies on this subject; in 1941, he died during one of the bombings of London.

Henceforth, the studies on transformation of pneumococci, which resulted in discovery of the key role of DNA in this process, were associated with the Rockefeller Institute for Medical Research (laboratory of Oswald Theodore Avery). By the beginning of 1930s, Avery (1877—1955) was already a recognized specialist in microbiology with numerous accomplishments in the field of immunochemistry of polysaccharides of pneumococci. Avery, in collaboration with Heidelberger, demonstrated that the pneumococci serotype is determined precisely by polysaccharides of their capsule; he began publishing articles on microbiology of pneumococci in 1915. He started working at the Rockefeller Institute in 1923. According to Avery's contemporaries (Hotchkiss, 1965; McCarty, 1994), he immediately noticed Griffith's publication, although he doubted the integrity of the experiment; he had concerns that not all serotype II cells died at 60°C. Avery confirmed the correctness of the conclusions in test experiments. Later, transformation in vitro (in liquid culture medium) was demonstrated in his laboratory instead of transformation in mice (Dauson and Sia, 1931; Sia and Dawson, 1931). Further, lysate obtained by pneumococci treatment with deoxycholate, precipitation with ethanol, and redissolution was used instead of dead cells. It maintained transforming activity (while with significant quantitative variations); i.e., it possessed a transforming factor (TF) of a yet unknown nature (Alloway, 1932, 1933). Although Avery led these studies, he was not featured as a coauthor; instead, he endorsed only the studies he personally participated in as an experimenter (Dubos, 1976). After 1933, his laboratory did not perform any studies of transformation of pneumococci for a long time.

Avery himself, in collaboration with Colin Munro MacLeod, began investigating the chemical nature of TF only eight years later; they were later joined by Maclyn McCarty, who had just entered the laboratory. According to McCarty's memoirs and other memoir literature sources (Dubos, 1976; McCarty, 1994), the events took place in the following order. During the spring of 1940, after summer vacation, Avery and MacLeod decided to begin a detailed study of TF. Autumn and winter were dedicated to experiments on deproteinization of the lysate by treating it with chloroform and isopropanol, which did not affect the activity of TF. In the early 1941, crystalline ribonuclease was demonstrated to have no effect on TF; and preparations containing TF also contained an agent that results in a color reaction with diphenylamine (Dische reaction, the assay of DNA). In the spring of 1941, the researchers reached replicable quantitative measurements of TF (due to modification of the cell destruction method by deoxycholate treatment). In the autumn of 1941, the hypothesis on polysaccharide origin of TF was rejected (polysaccharides of the capsule precipitated in a form of flakes after ethanol treatment of bacterial lysate; one of the working hypothesis allowed for a certain fraction of redissolved polysaccharides to be used as a primer for synthesizing similar polysaccharides upon introduction into the recipient cell). McCarty demonstrated complete conversion of polysaccharides into disaccharides by treating pneumococci lysate with hydrolase preparation from soil bacilli; it did not affect TF activity. In the early 1942, a viscous substance that was precipitated with ethanol in a form of strands, redissolved in saline, and gave positive results after being assayed with the Dische reaction was found to remain after the removal of proteins and polysaccharides. Its biological activity as a TF was extremely high. It was similar to DNA preparations (which at the time were extracted from mammalian tissue by Alfred Mirsky) in its physical and chemical properties. From this moment on, Avery's group assumed that TF is most probably identical to DNA, and searched for further proof for this assumption. The next half of 1942 was spent on treating TF preparations with various ferments. From winter to spring of 1943, after additional purification of preparations, the following became clear. TF was biologically active in extremely small quantities (after adding 3 ng of TF into the culture of decapsulated cells, large smooth colonies formed by encapsulated cells could still be detected); the carbon, phosphor and nitrogen contents of TF matched those of DNA; the Dische reaction with diphenylamine gave positive results. TF was resistant to treatment with trypsin, chymotrypsin, ribonuclease, and hydrolase, which degrade polysaccharides; the molecular mass of TF, as determined by ultracentrifugation, approached 500 thousand Da; the UV absorbency specter matched that of the DNA.

Finally, TF was susceptible to unpurified deoxyribonuclease preparations. Members of Avery's group shared their reasoning with Northrop and Stanley (these researchers were first to obtain crystalline preparations of pepsin and tobacco mosaic virus; these discoveries were for a certain time doubted). An encouraging answer was received. Two younger members of the group excitedly urged Avery to publish the results; "What else do you want, professor? What other evidence do you need?" asked MacLeod. Avery was still in doubt; he foresaw the arguments of the skeptics on the use of unpurified deoxyribonuclease preparations that could contain impurities of proteolytic ferments. These ferments could, in turn, degrade the "effective agent" of TF if it was of proteinic nature (some amount of proteins was indeed present in TF preparations). Nevertheless, in the autumn of 1943, the manuscript was written, the journal received it on November 1, and it was published in 1944 (Avery et al., 1944), when Avery was already 67. In parallel with this, McCarty obtained very pure deoxyribonuclease preparations from the pancreas. In 1946, two more manuscripts containing data on complete inactivation of TF from lysate of pneumococci were published (Avery et al., 1946a, 1964b).

The course of a further search for evidence of the role of DNA in heredity lies outside the field of transformation studies; they took place almost a decade later, making Avery's discovery ahead of its time. Even Dubos (Dubos, 1945), who was very familiar with Avery's work and Avery himself and subsequently wrote an apologetical book about him (Dubos, 1976), was confined to a detailed summary of the publication of 1944 without making any general conclusions on transformation through DNA, which is the transfer of genes; bacterial genetics in these years were only starting to develop. Avery himself avoided the term "gene," although such established geneticists as Lvov and Beadle noted as recently as 1948 changes of the polysaccharide types in experiments on transformation, which is similar to the transfer of allelomorphic genes that determine the synthesis of these polysaccharides (Dubos, 1976, p. 148, p. 156). In addition, other interests prevailed for many people in 1943, even in the relatively wealthy United States. As Coburn, who also worked with streptococci, including pneumococci, recalled (Coburn, 1969), Avery described to him in 1943 the unpublished results of the other study of his group in great detail and expressed confidence that TF is deoxyribonucleic acid. "My distracted answer followed: 'Is that so? Is this going to help us win the war?' My thoughts at the time were wandering to the battles with Rommel in North Africa and the Japanese fleet around the Coral Sea," writes Coburn (p. 626) (he could also mention the Battle of Stalingrad that had just ended by the time!—A.P.).

In 1944, Avery left the Rockefeller Institute and New York, and he passed away in 1955. He never met Griffith, but their mutual acquaintance Coburn gave Avery a photograph of this scientist, and Avery hung in at his work desk (Coburn, 1969). Avery's contemporaries named him the most worthy researcher not nominated for a Nobel Prize (although he was included in the list of nominees for his studies on the immunology of polysaccharides of pneumococci), and he was awarded a number of honorary awards on various occasions. McCarty, the last living coauthor of the famous publication of 1944, got the Lasker Award in 1994, together with Prusiner, for "DNA as the chemical substance of heredity" and "Prions as a cause of chronic neurodegenerative diseases" (Prusiner and McCarty, 2006).

FURTHER STUDIES ON GENETIC TRANSFORMATION OF PNEUMOCOCCI: MAIN EXPERIMENTAL TRENDS AND EMERGENCE OF NEW BACTERIAL MODELS

Further studies of pneumococci transformation (tentatively speaking, in 1950—1965) were mostly associated with the Rockefeller Institute for Medical Research and Rockefeller University (the laboratory of Hotchkiss, Avery's former employee). Selective markers (mainly resistance to antibiotics) were introduced into laboratory practice. This novelty significantly facilitated the experiments (the morphology of colonies in Avery's experiments was not a selective trait, and it required the inspection of numerous inoculated bacterial probes in order to find large smooth colonies of transformants among the small rough ones). The parameters for the dependence of the number of transformants on the DNA concentration (Hotchkiss, 1957) were determined, and other studies were performed that gave rise to whole new fields of study. First, the experiments demonstrated unequal ability to transform in pneumococcal cultures during different stages of growth (Hotchkiss, 1954). This observation began a great number of studies on the competence state during transformation in various bacteria. Next, new studies were published on the fate of transforming DNA within the pneumococcal cell that accepted it and its presence there in a polymer state (Lerman and Tolmach, 1957). During experiments on the reextraction of transforming DNA of the donor, it was found that this DNA assumes a so-called eclipse phase, in which it loses its transforming activity only to recover it again within the recipient cell's chromosome, within the first minutes after the uptake occurred (Fox and Hotchkiss, 1960). Lacks, a beginner student from the laboratory of Hotchkiss, proved that the eclipse phase corresponds to short-term, single-stranded state of the taken DNA (the second strand is destroyed upon entering the cell) while working with transforming DNA labeled with radioactive phosphor (Lacks, 1962). "That evening I had a moment of 'eureka,' writes Lacks in his memoirs about the moment when it was clear that almost half of all absorbed DNA that passed through the column was

situated within the area of single-stranded molecules; evidently, the other strand fragmented into small pieces the moment it was absorbed by the cell (Lacks, 2003, p. 2). By that time, it was already known that single-stranded DNA, which is generated during heated denaturation, possesses a very weak transforming ability (Marmur and Doty, 1961). Further studies on the competence and the fate of transforming DNA within the cell made it possible to gain a more complete picture of the multistage preparation of bacterial cell to the uptake of foreign DNA and its alteration within the recipient cell.

A phenomenon of so-called spontaneous transformation was described for pneumococci in Hotchkiss' laboratory (Ottolenghi and Hotchkiss, 1960) during joint growth of the recipient and donor cells; spontaneous transformation occurred due to the donors' DNA present in the culture liquid (the DNA was not added by the experimenter). The mechanism of transforming DNA entering the environment remained unknown for a long time. This study initiated research on bacterial transformation as a mean of horizontal gene transfer in the natural environments of microorganisms.

By the late 1950s–early 1960s, the "monopoly period" for pneumococci as the only model for transformation studies was over (this, however, did not mean that research on these bacteria has ended). Another simpler and unpretentious model was found—*Bacillus subtilis* (*B. subtilis* 168, or the Marburg strain, in particular). Spizizen included this bacillus in the group of transformed bacteria in 1958 (Spizizen, 1958); he, together with Anagnostopoulos, later published a number of studies. Auxotrophic mutants, which could be transformed into prototrophic ones, were soon obtained for *B. subtilis* 168. Auxotrophic mutants of this strain rapidly spread in various laboratories. In 1961, S.E. Bresler brought them into his laboratory in Leningrad (Leningrad Nuclear Physics Institute) from Paris. From there, the mutants came into the laboratory of R.B. Khesin in Moscow (Institute of Atomic Energy) in autumn of 1961. Khesin took an interest in this subject and published the first Russian paper, a review of studies on transformation (Hesin, 1958). Sometime earlier, in the middle of 1950s, another popular model for research on transformation was put in practice, *Haemophilus influenzae* (Alexander and Leidy, 1954). Researchers began using other bacteria as well (gonococci and meningococci). Later, the works on competence and the fate of the transforming DNA in the cell provided with the a full picture of the multi-stage preparation for the absorption of alien DNA and transformation of this DNA in the recipient cell. Thus by the early 1960s, prerequisites for detailed research on the state of competence in streptococci and other bacteria capable of transformation, as well as research on transformation as a method of DNA exchange in natural bacterial populations, were made.

RESEARCH ON THE STATE OF COMPETENCE IN STREPTOCOCCI

The state of cells during genetic transformation, in which they are able to adsorb transforming DNA, consume it, and incorporate it into the chromosome due to recombination, is called competence. As was mentioned earlier, pneumococci proved to be capable of transformation only at a particular stage of cultural growth (the early exponential phase), according to studies performed in the middle 1950s. A study performed on *S. grodoni* streptococci by Pakula in the early 1960s in Warsaw revealed that a certain substance appears in the middle of the exponential growth phase in cultural liquid; it later stimulates transformation in a culture "not yet matured" into the competence state (Pakula et al., 1962; Pakula, 1965). Similar works conducted on pneumococci were published roughly at the same time by Tomasz at Hotchkiss' and later his own laboratory (Tomasz and Hotchkiss, 1964; Tomasz, 1965). The substance was called "competence factor" in *S. gordoni* and "activator" in pneumococci.

Regulation of the competence state in pneumococci and other streptococci have been studied over the last fifty years in numerous laboratories; especially successful studies were conducted in the laboratories of Lacks (Brookhaven, United States), Morrison (Illinois, United States), and Havarstein (Norway), as well as Claverys (Toulouse, France). The competence was shown to be regulated by a large number of genes, which are divided into early and late genes. The early genes include approximately ten genes that "tune" the cell on acquiring competence (including *comM* gene; its product, the ComM protein, is situated on the surface of the cell and protects it from being destroyed by autolysins). Late genes include all other genes that determine the stages of the transformation itself: the DNA uptake, the changes in DNA during absorption by the cell, and recombination of the DNA with the chromosome. The course of events during the development of competence in pneumococcal culture can be very generally described as the following (it must be noted that particular parts of this scheme do not always match in descriptions of different authors). A product of the early competence gene *comC*, a precursor of the aforementioned activator (or competence factor) that was later denoted a competence pheromone, appears in the cell's cytosol at the very beginning of the growth of the culture. The pheromone's precursor is comprised of 45 amino-acid residues. It interacts with the peptide export system, which includes the products of *comAB* genes. During this time, the pheromone's precursor is cleaved within the leader sequence region, and a "matured" pheromone, a peptide 17 amino-acid residues long (from 14 to 23 residues in other streptococci; synthetic versions of all these pheromones were obtained), is released from the cell. Further, a two-component transferring system starts to act: the competence pheromone interacts

with its receptor on the cell's surface, the transmembrane portion of the histidine kinase, the product of the *comD* gene (sensor protein). Activated histidine kinase phosphorylates the product of *comE* gene (a protein that regulates response). This protein starts to accumulate in the cell. With an increase of the number of cells in the culture, the amount of pheromone increases in the environment. Thus, the amount of phosphorylated ComE protein increases in the cell as well; after reaching a certain concentration, this protein binds with promotor of the *comCDE* operon and, due to the positive regulation, stimulates its activity. In this manner, self-induction occurs and the cycle is complete: the more pheromone is released into the environment, the larger amounts of the ComE protein is phosphorylated, which stimulates the synthesis of new portions of pheromone. The "quorum sensing" envelops the population; the cells message each other in their "pheromone language": "there are many of us, so we can assume a new state; in this case, a state of competence" (Morrison, 1980; Lacks 1999; Havarstein and Morrison, 1999; Prozorov, 2001; Claverys et al., 2006; Johnsborg and Havarstein, 2009; Johnston et al., 2014a, 2014b; Straume et al., 2015). The functions of phosphorylated ComE protein are not limited to that; aside from stimulation of its promotor activity, it activates the synthesis of a product of *comX* gene. This gene in turn activates the synthesis of products of various other genes (more than a hundred) through their promotors. A number of these genes, which are categorized as late competence genes, are essential for further performing and completing the main stages of transformation. These stages include the adsorption of double-stranded DNA on the surface of recently discovered pilus of pneumococci (a "growth" of the cytoplasmic membrane in the form of a long strand protruding far beyond the borders of the cell that is comprised of pilin proteins); binding of this DNA with receptor of the cytoplasmic membrane, the ComEA protein; the "pulling in" and simultaneous destruction of one of the DNA strands in the cell by the EndA endonuclease; the passage of the DNA, now single-stranded, through the membrane's pore (which is formed by a ComEC protein); the interaction of the single-stranded DNA, which entered the cell's cytosol, with a "recombinase" protein RecA and "supporting" protein DprA (DNA processing proteinA); and the incorporation of the DNA within the cell's chromosome. The genes that control these processes form a hierarchy with epistatic interactions. In addition to the late competence genes, a substantial amount of other genes that were previously "silent" are activated; the products of these genes do not directly connect with the transformation processes. Various other chemicals that evoke cross linkage and other DNA damage can affect induction of the competence state; e.g., mitomycin C (Johnston et al., 2014b). Thus, the state of competence is sometimes suggested to be denoted as "phase X" for its polyfunctionality (Claverys et al., 2006).

It could be assumed that the cells that underwent a "competent development route" "set ready" all of the mechanisms necessary for a successful start and completion of transformation; it occurs independently of the occurrence of the future contact of these cells and transforming DNA (i.e., this can end in an "unproductive" manner: the cells always undergo the phase of readiness for DNA uptake; however, this readiness is realized on fairly rare occasions). An analogy with the mammalian ovulation cycle can be suggested here. However, as will be evident below, the formation of competent cells in streptococci is accompanied by the appearance of free DNA, which are released from incompetent specimens of the same population late in their development, in the culture. Thus, competent cells can always fulfill their "destiny" of consuming the DNA and incorporating it into their chromosome. The processes that result in extracellular DNA appearing in the streptococcal culture liquid will be researched in the next series of studies.

MECHANISMS OF DNA RELEASE FROM CELLS DURING NATURAL TRANSFORMATION AND THEIR ROLE IN HORIZONTAL GENE TRANSFER IN STREPTOCOCCI

As was mentioned above, the phenomenon first called spontaneous transformation was discovered in the Hotchkiss laboratory in 1960. While donor and recipient strains of pneumococci were growing together, recipient cells underwent transformation caused by the DNA released from the cells of donor strain. The application of DNase on the growing culture prevented this process. The term "spontaneous transformation" later became rarely used due to the identical mechanisms of DNA uptake by a competent cell when the DNA was released into the environment "naturally" or by the researcher during an experiment. Thus, in both cases the term "natural transformation" is usually used.

The DNA is released from the cells by autolysis processes. Almost all bacteria, including streptococci, possess autolytic ferments. Pneumococci, members of this group of microorganisms, possess at least three of these ferments: so-called major pneumococcal autolysin (LytA protein), murein hydrolase (LytC protein), and CbpD protein. Autolysins serve the same function in the cells of pneumococci and the cells of other bacteria: renewal of the peptidoglycan layer of the cell, involvement in cell division, and the isolation of the derived cells. But the most important function within the framework of present review is their involvement in the phenomenon of allolysis, the lysis of neighboring cells of the same species within the growing culture rather than their own cells (Claverys et al., 2006; Prozorov and Danilenko, 2011). It begs the question: do

the aforementioned state of competence in the growing culture of pneumococci and other streptococci and the allolysis of a portion of the cells occur at the same time accidentally or these events are connected by the same cause? For a while the evidence on this connection was only indirect. It was only at the beginning of this century a number of studies that obtained direct evidence of this connection were conducted in Havarstein's laboratory (Steinmoen et al., 2002, 2003). A strain of pneumococci with an inactivated early competence *comA* gene, which controls the competence pheromone release from the cell (see "Research on the State of Competence..." section), was studied. This strain was already devoid of competence, and its culture liquid did not contain any extracellular transforming DNA. However, after the addition of a synthetic pheromone into the culture, the cells became competent and DNA simultaneously appeared in the environment. This can only be explained by the lysis of a portion of the cells, which was induced by the pheromone. Approximately 20% of the cells of the culture were lysated. However, due to both competent and incompetent cells present in the culture, it was necessary to determine the fraction the lysated cells belonged to (or whether they were equally distributed among both fractions). The answer was obtained during the following experiments. Two pneumococcal strains were used; one carried a mutation in the *comA* gene; another had an additional mutation of another early competence gene, *comE* (it lacked the protein that regulates response). This strain was resistant to novobiocin. The absence of ComE protein was not compensated by an addition of pheromone into the environment. The strains were grown separately; the cultures were then mixed and the competence pheromone was added into the environment. It could inducethe development of competence only in strains with the *comA* mutation. Free DNA with a marker of resistance to novobiocin appeared in this mixed culture. This meant that only cells unable to develop competence were lysated, and their lysis was directly connected to the induction of competence in the partner strain. It was discovered later that mainly the ClpD protein was responsible for lysis (Wei and Havarstein, 2012). It must be noted that the competent cells were protected from their own lytic ferments due to the ComM and CibC proteins (also products of the early competence genes; incompetent cells lacked these proteins and were defenseless against the complex of lytic ferments) situated on the cell wall. The lysis of incompetent cells occurred during direct contact with the competent "killer" cells (which was accompanied by agglutination). As a result, the DNA was released from destroyed bacteria and immediately consumed by the competent cells. Similar results were obtained almost simultaneously in Claverys' laboratory (Moscoso and Claverys, 2004; Claverys and Havarstein, 2006).

Thus, competent cells appearing in a culture of pneumococci, the destruction of still incompetent bacteria, the release of their DNA, and the uptake of this DNA by the competent cells have proven to be consecutive stages and not accidentally matching occurrences.

Allolysis is virtually a form of cannibalism in bacteria. To denote the fact that killed incompetent cells do not differ from their competent "killers," the referenced papers introduced the term "fratricide" (from the Latin word *frater*, "brother"). The ferments used to perform fratricide were denoted fratricins (Berg et al., 2012). The DNA released outside the destroyed cell and immediately taken up by the competent bacteria of the same strain does not bring anything new into the recipient's genome (apart from the alleles, which were altered during the construction of partner strains: resistance to novobiocin, etc), although it contributes to the creation of the merodiploids (Johnston et al., 2013). However, allolysis was also described during the combined growth of pneumococci and closely related streptococci species (e.g., *S. mitis* and *S. oralis*). In this case, this process is called sobrinicide (the killing of relatives; from the Latin word *sobrinus*, cousin). Natural transformation of streptococci is a mixture of cannibalism (fratricide), predation (sobrinicide), and an analog of a sexual process (uptake of the DNA of a partner cell). In other words, competent cells kill their neighbors in order to uptake their DNA and "fertilize themselves" by inserting this DNA into their own genome. Apparently, DNA is released in the environment not only by means of allolysis but also during various other autolytic processes. The release of transforming DNA (not only chromosomal but plasmid DNA as well) from the cells is described in *B. subtilis* (Takahashi, 1962; Prozorov and Glumova, 1980), a bacterium with "temporary" competence; in meningococci (Catlin, 1960); and in other microorganisms. The phenomenon of transformation is one of the numerous routes of horizontal gene transfer for the environmental bacteria.

The human oral cavity and nasopharynx are such environments for streptococci. They form biofilms linked by polysaccharides of the capsules on the dental plaque, tonsils, and nasopharyngeal epithelium (Li et al., 2001). Various species of commensal and opportunistic streptococci inhabit the biofilms aside from *S. pneumoniae*: *S. mutans*, *S. mitis*, *S. oralis*, *S. gordoni*, *S. sanguis*, *S. anginosus*, etc. Due to fratricide and sobrinicide, gene transfer frequently occurs from commensal to pathogen species, including pneumococci. Strains of pneumococci with "foreign" genes of penicillin resistance were isolated. The researchers such as Hakenbeck (first at the Rockefeller Institute, later at the institutes of the Federal Republic of Germany) performed an especially large number of studies on this subject (Martin et al., 1992; Havarstein et al., 1997; Henriques-Normark et al., 2008). The genes that determine pneumococcal serotypes are transferred within the natural populations in the same manner. Because there are currently more than 90 dif-

ferent known encapsulated serotypes of pneumococci, constantly appearing new serotypes complicate the use of preexisting vaccines active against the seven main serotypes of these bacteria (Henriques-Normark et al., 2008). Certain studies (Hiller et al., 2007; Johnsborg et al., 2008) hypothesize about the idea of a common "supergenome" for all streptococci of the biofilms in the oral cavity and nasopharynx.

CONCLUSIONS

To this day, pneumococci can be considered leaders in the extent to which the transformation processes are studied in an organism (Johnston et al., 2014a). Nevertheless, natural transformation was described for more than 82 bacterial species (Johnston et al., 2014b); a particularly high number of studies were performed on *B. subtilis*. Unique features of this process are present in nearly every species. This includes even such a "fundamental" stage as the presence of the state of competence. In most bacterial species with the ability to undergo transformation, the competence state is temporary for a growing microbial culture; however, *Neisseria* (gonococci and meningococci) and possibly *Helicobacter pylori* have so-called constitutive competence, during which cells can uptake transforming DNA at all times. The processes of acquiring competence are strongly associated with sporogenic stages and are regulated by the same genes in *B. subtilis*, which substantially complicate (like some nightmare—D. Dubnau's remark) the gene nomenclature in this bacterium. Thus, it is hard to reduce all unique features of transformation in all microorganisms to the same denominator. However, during the last 10–15 years, a number of studies on the homology of proteins involved in transformation in various bacteria with different degrees of relationship appeared due to the mass sequencing of bacterial genomes and the rapid development of comparative genomics and proteomics. A detailed analysis of these studies was given in a recently published review (Johnston et al., 2014b). A thesis was formulated: in phylogenetically distant bacterial species, the proteins involved in DNA adsorption on the cell, in its uptake, and especially in its further transformation up to the point of recombination with the recipient's chromosome, are relatively conservative and very similar to each other. On the contrary, the mechanisms regulating the initiation and termination of different transformation stages can differ even in closely related species. For example, the pheromones of competence substantially differ in size and structure (from 14 to 23 amino-acid residues) in pneumococci and other streptococci. Streptococci also have differences in the mechanisms of activation of the cluster of late competence genes by *comX* gene (Kaspar et al., 2015).

Since natural transformation is widespread among bacteria, this phenomenon can be considered a form of sexual process. Every species possesses specific features of its mechanism of transformation, which were studied in the most detail in pneumococci. Nevertheless, the basis and a distinct feature of the transformation process is the uptake of a "naked" DNA strand, which is carried out without the involvement of mobile elements, plasmids, transposons, and bacteriophages. It was discovered for the first time more than seventy years ago, during experimentation by Avery and his colleagues.

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REFERENCES

Alexander H. and Leidy G. Induction of streptomycin resistance in sensitive *Haemophilus influenzae* by extracts containing DNA from resistant *Haemophilus influenza*, *J. Exp. Med.*, 1954, vol. 97, no. 6, pp. 706–806.

Alloway, J., The transformation in vitro of R pneumococci into S forms of different specific types by the use of filtered *Pneumococcus* extracts, *J. Exp. Med.*, 1932, vol. 55, no. 6, pp. 91–99.

Alloway, J., Further observations on the use of pneumococcus extracts in effecting transformation of type in vitro, *J. Exp. Med.*, 1933, vol. 57, no. 2, pp. 265–278.

Avery, O., MacLeod, C., and McCarty, M., Studies on the chemical nature of the substance inducing transformation of pneumococcal types. I. Induction of transformation by a deoxyribonucleic acid isolated from pneumococcus type III, *J. Exp. Med.*, 1944, vol. 79, no. 1, pp. 137–157.

Avery, O., McCarty, M., and MacLeod, C., Studies on the chemical nature of the substance inducing transformation of pneumococcal types. II. Effects of deoxiribonuclease on the biological activity of the transforming substance, *J. Exp. Med.*, 1946a, vol. 83, no. 1, pp. 89–96.

Avery, O., McCarty, M., and MacLeod, C., Studies on the chemical nature of the substance inducing transformation of pneumococcal types. III. An improved method for the isolation of the transforming substance and its application to pneumococcal types II, III, and IV, *J. Exp. Med.*, 1946b, vol. 83, no. 1, pp. 97–109.

Baurhenn, W., Experimentelle Untersuhungen zur Variabilität und zur Analyse der R-S Umvondlurg von Pneumokokken, mit besonders Berücksichtigurg der Grappe, *Cent. Bacteriol.*, 1932, vol. 126, no. 1, pp. 68–92.

Berg, K., Biornstad, T., Johnsborg, O., et al., Properties and biological role of streptococcal fratricins, *Appl. Environ. Microbiol.*, 2012, vol. 78, no. 10, pp. 3515–3522.

Catlin, B., Transformation of *Neisseria meningitidis* by deoxyribonucleates from cells and from culture slime, *J. Bacteriol.*, 1960, vol. 79, no. 3, pp. 579–590.

Claverys, J.P. and Havarstein, L., Cannibalism and fratricide: mechanisms and raison d'être, *Nat. Rev. Microbiol.*, 2006, vol. 5, no. 3, pp. 219–229.

Claverys, J.P., Prudhomme, M., and Martin, B., Induction of competence regulons as a general response to stress in gram-positive bacteria, *Annu. Rev. Microbiol.*, 2006, vol. 60, pp. 451–475.

Coburn, A., Oswald Theodore Avery and DNA, *Persp. Biol. Med.*, 1969, vol. 12, no. 4, pp. 623–630.

Dawson, M. and Sia, R., In vitro transformation of pneumococcal types. I. A technique for inducing transformation of pneumococcal types in vitro, *J. Exp. Med.*, 1931, vol. 54, no. 4, pp. 681–699.

Dubos, R.J., *The Bacterial Cell in its Relation to Problems of Virulence, Immunity, and Chemotherapy*, Cambridge, MA: Harvard Univ. Press, 1945.

Dubos, R.-J., *The Professor, the Institute, and DNA*, New York: Rockfeller Univ. Press, 1976.

Fox, M. and Hotchkiss, R., Fate of transforming deoxyribonucleate following fixation by transformable bacteria, *Nature*, 1960, vol. 187, pp. 1002–1006.

Griffith, F., The significance of pneumococcal types, *J. Hyg.*, 1928, vol. 27, no. 2, pp. 113–119.

Havarstein, L., Hakenbeck, R., and Ganstad, P., Natural competence in the genus *Streptococcus*: evidence that streptococci can change pherotype by interspecies recombinational exchanges, *J. Bacteriol.*, 1997, vol. 179, no. 21, pp. 6589–6594.

Havarstein, L. and Morrison, D., Quorum sensing and peptide pheromones in streptococcal competene for genetic transformation, in *Cell-Cell Signaling in Bacteria*, Dunny, C. and Winans, S., Eds., Washington, DC: Am. Soc. Microbiol., 1999, pp. 9–26.

Henriques-Normark, B., Blomberg, C., Dagerhamn, J., et al., The rise and fall of bacterial clones: *Streptococcus pneumoniae*, *Nat. Rev. Microbiol.*, 2008, vol. 6, no. 11, pp. 827–837.

Hiller, N., Janto, B., Hogg, J., et al., Comparative genomic analyses of seventeen *Streptococcus pneumoniae* strains: insights into the pneumococcal supragenome, *J. Bacteriol.*, 2007, vol. 189, no. 22, pp. 8186–8195.

Hotchkiss, R., Cyclical behavior in pneumococcal growth and transformability occasioned by environmental changes, *Proc. Natl. Acad. Sci. U.S.A.*, 1954, vol. 40, no. 2, pp. 49–55.

Hotchkiss, R., Criteria for the quantitative genetic transformation of bacteria, in *The Chemical Basis of Heredity*, McElroy, W. and Glass, B., Eds., Baltimore, MD: Johns Hopkins, 1957, pp. 321–335.

Hotchkiss, R. Oswald T. Avery, 1877–1955, *Genetics*, 1965, vol. 52, no. 1, pp. 1–10.

Johnsborg, O., Eldholm, V., and Bjørnstad, M., A predatory mechanism dramatically increases the efficiency of lateral gene transfer in *Streptococcus pneumoniae* and related commensal species, *Mol. Microbiol.*, 2008, vol. 69, no. 1, pp. 245–253.

Johnsborg, O. and Havarstein, L., Regulation of natural genetic transformation and acquisition of transforming DNA of transforming DNA in *Streptococcus pneumoniae*, *FEMS Microbiol. Rev.*, 2009, vol. 33, no. 3, pp. 627–642.

Johnston, C., Campo, N., Bergé, M., et al., *Streptococcus pneumoniae*, le transformiste, *Trends Microbiol.*, 2014a, vol. 22, no. 3, pp. 113–119.

Johnston, C., Caymaris, S., Zomer, A., et al., Natural genetic transformation generates a population of merodiploids in *Streptococcus pneumoniae*, *PLoS Genet.*, 2013, vol. 9, no. 9, p. e1003819.

Johnston, C., Martin, B., Fichant, G., et al., Bacterial transformation: distribution, shared mechanisms and divergent control, *Nat. Rev. Microbiol.*, 2014b, vol. 12, no. 3, pp. 181–196.

Kaspar, J., Ahn, S., Palmer, S., et al., A unique open reading frame within the *comX* gene of *Streptococcus mutans* regulates genetic competence and oxidative stress tolerance, *Mol. Microbiol.*, 2015, vol. 96, no. 3, pp. 463–482.

Khesin, R.B., Role of deoxyribonucleic acids in bacterial transformation, *Usp. Sovrem. Biol.*, 1958, vol. 46, no. 2, pp. 113–129.

Lacks, S., Molecular fate of DNA in genetic transformation of *Pneumococcus*, *J. Mol. Biol.*, 1962, vol. 5, no. 1, pp. 119–131.

Lacks, S., DNA uptake by transformable bacteria, in *Transport of Molecules Across Microbial Membranes*, Broome-Smith, J.K., Baumberg, S., Stirling, C.J., , Eds., Cambridge, UK: Cambridge Univ. Press, 1999, pp. 139–188.

Lacks, S., Rambling and scrambling in bacterial transformation—a historical and personal memoir, *J. Bacteriol.*, 2003, vol. 185, no. 1, pp. 1–6.

Lerman, L. and Tolmach, L., Genetic transformation. I. Cellular incorporation of DNA accompanying transformation in *Pneumococcus*, *Biochim. Biophys. Acta*, 1957, vol. 26, no. 1, pp. 68–82.

Li, Y., Lau, P., Lee, J., et al., Natural genetic transformation of *Streptococcus mutans* growing in biofilms, *J. Bacteriol.*, 2001, vol. 183, no. 3, pp. 897–908.

Marmur, J. and Doty, P., Thermal denaturation of deoxyribonucleic acid, *J. Mol. Biol.*, 1961, vol. 3, no. 5, pp. 585–594.

Martin, C., Sibold, C., and Hakenbeck, R., Relatedness of penicillin-binding protein 1a genes from different clones of penicillin-resistant *Streptococcus pneumoniae* isolated in South Africa and Spain, *EMBO J.*, 1992, vol. 11, no. 11, pp. 3831–3836.

McCarty, M., A retrospective look: how we identified the pneumococcal transforming substance as DNA, *J. Exp. Med.*, 1994, vol. 179, no. 2, pp. 385–394.

Morrison, D., Competence-specific protein synthesis in *Streptococcus pneumoniae*, in *Transformation-1980*, Polsinelli, M. and Mazza, G., Eds. Oxford: Cotswold, 1980, pp. 39–54.

Moscoso, M. and Claverys, J., Release of DNA into the medium by competent *Streptococcus pneumoniae*: kinetics, mechanism and stability of the liberated DNA, *Mol. Microbiol.*, 2004, vol. 54, no. 3, pp. 783–794.

Neufeld, F. and Levinthal, W., Beitrage zur Variabilitat der Pneumokokken, *Z. Immunitätsforsch.,* 1928, vol. 55, no. 3, pp. 324–340.

Ottolenghi, E. and Hotchkiss, R., Appearance of genetic transforming activity in pneumococcal cultures, *Science*, 1960, vol. 132, no. 3435, pp. 1257–1258.

Pakula, R., Production of competence-provoking factor and development of competence of a transformable

streptococcus in serum-free media, *Can. J. Microbiol.*, 1965, vol. 11, no. 5, pp. 811–822.

Pakula, R., Piechowska, M., Bankowska, E., et al., A characteristic of DNA mediated transformation systems of two streptococcal strains, *Acta Microb. Pol.*, 1962, vol. 11, no. 3, pp. 205–221.

Prozorov, A.A., *Transformatsiya u bakterii (Bacterial Transformation)*, Moscow: Nauka, 1988.

Prozorov, A.A., Competence pheromones in bacteria, *Microbiology* (Moscow), 2001, vol. 70, no. 1, pp. 1–9.

Prozorov, A.A. and Danilenko, V.N., Allolysis in bacteria, *Microbiology* (Moscow), 2011, vol. 80, no. 1, pp. 1–9.

Prozorov, A. and Glumova, E., Transfer of pUB110 plasmid via transformation in *Bacillus subtilis*, *Plasmid*, 1980, vol. 3, no. 1, pp. 231–232.

Prusiner, S. and McCarty, M., Discovering DNA encodes heredity and prions are infectious proteins, *Annu. Rev. Genet.*, 2006, vol. 40, pp. 25–45.

Reimann, H., The reversion of R to S pneumococci, *J. Exp. Med.*, 1929, vol. 49, no. 3, pp. 237–249.

Sia, R. and Dawson, M., In vitro transformation of *Pneumococcal* types: II. The nature of the factor responsible for the transformation of *Pneumococcal* types, *J. Exp. Med.*, 1931, vol. 54, no. 4, pp. 701–708.

Spizizen, J., Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate, *Proc. Natl. Acad. Sci. U.S.A.*, 1958, vol. 44, no. 10, pp. 1072–1078.

Steinmoen, H., Knutsen, E., and Havarstein, L., Induction of natural competence in *Streptococcus pneumoniae* triggers lysis and DNA release from a subfraction of the cell population, *Proc. Natl. Acad. Sci. U.S.A.*, 2002, vol. 99, no. 11, pp. 7681–7686.

Steinmoen, H., Teigen, A., and Havarstein, L., Competence-induced cells of *Streptococcus pneumoniae* lyse competence-deficient cells of the same strain during co-cultivation, *J. Bacteriol.*, 2003, vol. 185, no. 24, pp. 7176–7183.

Straume, D., Stamsas, G., and Havarstein, L., Natural transformation and genome evolution in *Streptococcus pneumoniae*, *Infect. Genet. Evol.*, 2014. doi 10.1016/j.meegid.2014.10.020

Takahashi, I., Genetic transformation of *Bacillus subtilis* by extracellular DNA, *Biochem. Biophys. Res. Commun.*, 1962, vol. 7, no. 6, pp. 467–470.

Tomasz, A. and Hotchkiss, R., Regulation of the transformability of pneumococcal cultures by macromolecular cell products, *Proc. Natl. Acad. Sci. U.S.A.*, 1964, vol. 51, no. 3, pp. 480–486.

Tomasz, A., Control of the competent state in *Pneumococcus* by a hormone-like cell product: an example for a new type of regulatory mechanism in bacteria, *Nature*, 1965, vol. 208, no. 5006, pp. 155–159.

Wei, H. and Havarstein, L., Fracticide is essential for efficient gene transfer between pneumococci in biofilms, *Appl. Environ. Microbiol.*, 2012, vol. 78, no. 16, pp. 5897–5905.

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