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THE STATE OF ART AND PROSPECTS FOR DEVELOPMENT OF SYMBIOGENETICS

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❀ The modern stage of development of symbiogenetics, a biological discipline that addresses the formation of super-species genetic systems, is associated with the study of molecular mechanisms and environmental consequences of combining the hereditary factors of prokaryotes and eukaryotes into functionally integrated symbiogenomes, which, as partners lose their ability to autonomous existence, are transformed into structurally integrated hologenomes. The loss by intracellular symbionts of eukaryotes of their genetic individuality, determined by the ability to independently maintain and express the genome, representing a key step in symbiogenesis which results in the transformation of bacteria into cellular organelles. Genetic reconstruction of symbiogenesis provides the broad prospects for its artificial reproduction aimed at the synthesis of new organisms and biosystems possessing the predetermined sets of practically significant features.

❀ **Keywords:** symbiogenetics; interactions of prokaryotes and eukaryotes; theory of symbiogenesis; hologenome and symbiogenome; symbiotic nitrogen fixation; intracellular symbionts; symbiosomes and cellular organelles; synthetic biology.

СОВРЕМЕННОЕ СОСТОЯНИЕ И ПЕРСПЕКТИВЫ РАЗВИТИЯ СИМБИОГЕНЕТИКИ

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❀ Современный этап развития симбиогенетики — биологической дисциплины, изучающей формирование надорганизменных генетических систем, — связан с изучением молекулярных механизмов и экологических последствий объединения наследственных факторов прокариот и эукариот в функционально интегрированные симбиогеномы, которые по мере утраты партнерами способности к автономному существованию преобразуются в структурно интегрированные хологеномы. Потеря внутриклеточными симбионтами эукариот генетической индивидуальности, определяемой способностью к самостоятельному поддержанию и экспрессии генома, является ключевым этапом симбиогенеза и знаменует преобразование бактерий в клеточные органеллы. Генетическая реконструкция симбиогенеза открывает широкие перспективы для его искусственного воспроизведения, которое направлено на синтез новых организмов и биосистем, обладающих заданным комплексом практически значимых признаков.

❀ **Ключевые слова:** симбиогенетика; взаимодействие прокариот и эукариот; теория симбиогенеза; хологеном и симбиогеном; симбиотическая азотфиксация; внутриклеточные симбионты; симбиосомы и клеточные органеллы; синтетическая биология.

INTRODUCTION

This issue of the journal is devoted to the current issues in symbiogenetics, a new and actively developing science of heredity, which studies the genetic integration of organisms in interspecies interaction systems. Historically, symbiogenetics originated with the development of facultative microbial and plant interactions models [1], which enabled the study of molecular mechanisms underlying functional integration of prokaryotic and eukaryotic genes. This is based on their signal interactions, the formation of integrated metabolic pathways, and in some cases the development of new tissue and cellular structures. The description of these processes within the framework of the principle of complementarity of genomes [2] enabled linkage to the complementary interaction of prokaryotic and eukaryotic genes with the expansion of their adaptive potential based on the development of "emergent" (missing outside of symbiosis) signs.

The use of obligate symbiosis models, as along with parasitic systems, expanded the range of the molecular processes analyzed. These processes are associated with the formation of supraorganismal gene systems (SOGS) at different biological organization levels [3, 4]. The discovery of the evolutionary history of intracellular symbionts (endocytobionts) of insects [5] and protozoal photosynthetic symbionts (chromatophores and cyanellas [6]) resulted in the reconstruction of symbiogenesis as a fundamental process that determined the emergence and further evolution of eukaryotes. Consequently, modern eukaryotic genomes can be represented as chimeric gene networks formed by prokaryotic organisms over billions of years of joint evolution. These gene networks retained the basic property of the prokaryotic genome, which is the division into a strictly vertically inherited cortex represented by the host's nuclear genome, and a much less strictly inherited accessory portion represented by the metagenome of the symbiotic microbial community.

SUPRASPECIFIC GENETIC SYSTEMS

Eukaryotic organisms that emerged 1.5–2 billion years ago because of a combination of bacteria and archaea continued to evolve as supraorganismal complexes, recruiting new types of microsymbionts into their structure. The most complex multicomponent systems, termed holobionts [7], are represented by multicellular eukaryotes that form various intra- and intercellular compartments for maintaining microorganisms. Symbiotic microorganisms receive nutrition and protection from their hosts in an unstable external environment. They compensate for the insufficient biochemical "repertoire" of eukaryotes, which relieves them from the requirement to maintain the nitrogen fixing and chemosynthesis gene systems, the formation of essential metabolites (amino acids, cofactors, and vitamins), and degradation of biopolymers, for example, cellulose and pectin, which form the basis of nutrition in most animals.

Currently, the symbiotic activity of eukaryotes is studied within the concept of *hologenome*, a combination of hereditary factors of the eukaryotic host and the microsymbionts inhabiting it [8]. Because of the extraordinary heterogeneity of genetic interactions between partners and for a more detailed characterization of SOGS, this concept was supplemented with the notion of *symbiogenome*, a system of functionally interrelated hereditary factors that interact directly with the development of symbiosis but do not manifest with the individual adaptation of partners [9]. The formation of a structural system of heredity determines the transformation of symbiogenome into hologenome. This process begins with the inclusion of symbionts in the reproductive cycle of the host and ensures the permanent maintenance of SOGS. Notably, the host's nuclear genome functions as a conservative cortex in the structure of SOGS, providing "housekeeping" of the cell; however, the microbial community metagenome represents the rapidly changing accessory portion that determines the host's adaptation to an unstable external environment. This structure of SOGS eliminates the need of eukaryotes to maintain extensive gene systems "for every occasion;" instead, a limited number of microbial hosting genes are used for operational adaptations to specific environmental situations.

The evolution of SOGS is best illustrated in the study of insect symbionts, which shift from facultative dependence on the host, usually restricted to colonization of extracellular niches in the intestine or hemolymph, to obligatory dependence associated with intracellular existence and intergenerational transmission of symbionts through host reproduction [5]. The complete loss of autonomous existence, which is typical for the majority of insect endocytobionts, is associated with the reduction of a significant portion (sometimes >90%) of the genome. A majority of these bacteria retain the ability to maintain and express their "minimal" genome; however, in some cases there is a notable simplification of the control systems of matrix processes, which further enhances the host dependence of symbionts [10].

Nevertheless, the integration of partners' heredity systems obviously results in the loss of their genetic individuality, which is recorded at two levels, autonomous existence and independent maintenance of the genome. The loss of some functions of autonomous existence has already been traced in facultative plant symbionts. Thus, the conversion of free-living nitrogen fixers related to *Rhodopsudomonas* into primary rhizobia (*Bradyrhizobium*) was associated with a loss of phototrophicity (bacteria switches to nutrition from products of plant photosynthesis) and diazotrophicity (the expression of the nitrogenase genes is limited by symbiosis and is aimed solely at supplying nitrogen to the host) [11].

The transfer of sym-genes from primary rhizobia to various soil and plant-associated microorganisms, including phytopathogenic microorganisms resulted in the subsequent emergence of secondary rhizobia (*Rhizobium*, *Sinorhizobium*, *Neorhizobium*, and *Mesorhizobium*) [11]. The deep specialization of secondary rhizobia to symbiotrophic carbon nutrition is associated with the bacterial transformation into bacteroids that are unable to propagate; this determined the transition to the "altruistic" strategy of symbiosis [12]. Notably, numerous genes that are similar to plant protection factors against phytopathogens are involved in the regulation of highly efficient symbiosis. This indicates a close evolutionary relation between the mechanisms of partner integration in mutualistic and antagonistic symbiosis.

FROM BACTERIA TO ORGANELLES

The qualitative difference between organelles and their predecessors, endocytobionts inherited by intergenerational transmission, is because of their loss of genetic individuality determined by the ability to independently maintain and express the genome. The first signs of this loss were detected in genetically simplified insect symbionts. For example, these bacteria supply hosts (aphids [*Buchnera*]) with essential amino acids and do not have several DNA reparation and recombination genes and most σ subunits of RNA polymerase [10]. Further reduction of matrix processes, typical for organelles, is associated with the transfer of their hereditary material to the host nuclear chromosomes through endosymbiotic gene transfer (EGT) [14]. Most organelles are characterized by the complete absence of DNA replication genes, and mitochondria are further characterized by transcription genes. This loss can be symbolized as a transition from the reduced to the rudimentary genome, which cannot function independently. This process is only possible if there is a constant import of enzymes that replicate and transcribe, along with various components of the translation apparatus (tRNA, ribosomal proteins, and aminoacyl-tRNA synthetase); however, a significant portion of this apparatus remains in organelles.

The initial stages of gene redistribution between symbiotic partners were studied using chromatophores of the protozoan *Paulinella chromatophora*, which is characterized by a relatively small degree of reduction; approximately 30% of the genome characteristic of the ancestor of chromatophores, unicellular cyanobacterium *Synechococcus*, is retained [6]. In this system, the transfer of only a few genes encoding the components of photosystem 1 from the endosymbiont to the host was discovered; however, the transfer of genes encoding matrix processes remains unknown. Thus, during symbiogenesis, the redistribution of genetic material between SOGS components begins with the "operational" genes encoding energy metabolism and is only later extended to the "informational" genes encoding matrix processes.

An important stage of symbiogenesis is the formation of symbiosomes, which are stable intracellular compartments containing microsymbionts [13]. The study of legume-rhizobia symbiosis enabled the tracing of the evolution of these compartments from unspecialized symbiosomes, in which bacteria completely retain their viability, to specialized symbiosomes (each of which contain one morphologically modified bacteroid). In the latter case, in symbiosomes, the space between the pro- and eukaryotic membranes is considerably reduced, similar to that in organelles; however, only relatively simple C- and N-metabolites are transported between the partners. Although bacteroids retain the full-size genome, they are characterized by loss of reproductive activity determined by the genes of both partners. Interestingly, the genetically reduced *Buchnera* symbionts, which have lost >80% of the genome and are incapable of autonomous existence, retain the ability to divide in the symbiosomes formed in insect host cells [10].

The formation of genome-free organelles, including mitochondrial derivatives such as mitosomes and hydrogenosomes of the anaerobic protozoa and plastids of the unicellular algae *Polytomella* closely related to Chlamydomonas can be considered to be the culmination of symbiogenesis. [14, 15]. Nevertheless, even with complete loss of the genome, organelles remain cellular entities that can reproduce and implement complex biochemical programs.

Thus, in the study of symbiogenesis, we encounter the "phenotype without genotype" phenomenon, which is unknown to classical genetics, and in particular, has the possibility of the manifestation of basic life functions, such as reproduction and metabolism, in the absence of systems for storing and expressing hereditary information encoding these functions. Interestingly, the reduction in the evolution of organelles can be considered to be a return of bacteria to the ancestral forms of cell organization. Thus, increased evolutionary stability of ribosomal protein synthesis compared with DNA-dependent matrix processes (replication, recombination, reparation, and transcription) can be considered to confirm the hypothesis regarding the conversion of RNA genomes to DNA genomes, which occurs at precellular (viral) and cellular levels [16].

IS SYMBIOGENESIS POSSIBLE *IN VITRO*?

The widespread occurrence of genetic integration between unrelated organisms results in the possibility of the utilization of this phenomenon in designing new biosystems. The first step toward the implementation of this idea is improving the existing symbiosis, which already occurs in microbial and plant relations providing nourishment and protection to crops. Specifically, to improve the efficiency of legume-rhizobia symbiosis, a genetic algorithm was proposed; this included enhancing the basic function of nitrogen fixation symbiosis and inactivating negative regulators of this function, which determine the ability of rhizobia to convert the carbon derived from plants into reserve food material and assimilate "non-symbiotic" (not used for bacteroid nourishment) carbon sources and synthesize protective components of the cell surface [17].

The development of new nitrogen-fixing organelles based on mitochondria and plastids, with ancestors (α-proteobacteria of the orders *Rhizobiales* and *Rickettsiales*, as along with cyanobacteria related to *Nostoc*) that were capable of fixing nitrogen or had nitrogen-fixing congeners, is promising for increasing the contribution of biological nitrogen to agricultural production [18, 19]. To date, the expression of certain *nif*-genes in yeast mitochondria with the formation of functionally active protein products has been demonstrated [20], which confirms the compatibility of the genetic background of organelles with nitrogenase complex synthesis.

Another approach to increase the contribution of nitrogen fixation in crop production involves transferring the nodule formation genes from legumes to cereals. Although the possibility of maintaining these genes in an alien environment has already been established, the likelihood of their complete phenotypic manifestation requires further study [21]. Roots of wheat and maize under the influence of the auxin analog 2,4D, nodule-like structures have been reported to appear and are accessible for colonization by azospirillum. Rhizospheric diazotrophs have a growth-promoting effect mainly due to phytohormone synthesis because most of the nitrogen fixed in the rhizosphere passes into the soil. However, on entering the artificially induced nodules, the nitrogenase activity of azospirillum markedly increases, and its products enter the plant almost completely [22]. There is evidence to support the use of this system for modeling the evolution of nodule symbiosis. Thus, symbiotic genes of plants and bacteria involved in their recognition and signaling interaction are not absolutely essential for the penetration of rhizobia into plants, and the presence of these genes only stabilizes the development of symbiosis and increases its functioning efficiency [23].

Further development of symbiotic engineering approaches is possible based on synthetic biological methods combining computer modeling of new genomes and their subsequent chemical synthesis. Intracellular symbionts and eukaryotic organelles that have lost the main portion of the genome but have retained the ability to reproduce and metabolize may be suitable recipients for their maintenance. This approach was successfully implemented by synthesizing the genome of *Mycoplasma mycoides* (1080 kbp), which was introduced into another genetically reduced bacterium *M. capricolum* [24]. Natural organelles, which are adapted to continuous existence in eukaryotic cells and have ample opportunities to express foreign genes and regulate the function their products, can be even more promising for the implementation of synthetic genomic approaches. Consequently, the use of symbiogenetic approaches provides extensive prospects for the development of new genetic technologies that enable the creation of organisms with a predetermined set of practically significant properties.

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