Genetics

Lateral transfer of genetic information

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From the original findings of genes persists a dogma suggesting that each organism has only one, i.e., its own, genome. Also persistent was the assumption that every evolutionary mechanism and development of new species was the result of gene recombination and/or new genetic mutations transferred vertically, from parents to offspring. The idea of endosymbiosis as a co-existence of taxonomically distinct organisms emerged more than 100 years ago. It was based on microscopic observations showing that some organisms are contained inside other organisms. These observations suggested that during evolution organisms were able to incorporate (both inside their internal milieu and inside their cells) organisms with different genomes, using direct horizontal processes across the species barriers. Numerous subsequent studies brought direct proof that this horizontal transfer of genes is quite common among microbes. Similarly, clear proof exists that these processes also occurred in many currently living multicellular organisms. More and more biologists now believe that horizontal transfer of genetic information significantly influenced formation of new characteristics and subsequently an evolution of new species. *Journal of Nature and Science, 1(4):e87, 2015*

Symbiosis | genome | evolution | bacteria | DNA | nucleus

First studies

In the middle of the 19th century some scientists started to believe that at least some multicellular organisms are in fact mosaics of several individual organisms. However, these hypotheses were often ridiculed and never gained mainstream acceptance. Swiss botanist S. Schwendener suggested in 1867 that lichen can have double character and hypothesized the possible evolutionary reasons for this type of co-existence (Schwedener , 1867). De Bary later pushed this concept further (1878, 1879) when he coined the concept of symbiosis to designate the type of coexistence of two non-related organisms: dividing the relationships between individual organisms into several degrees, from free co-existence to primitive cooperation, competition, parasitism, and predation. The idea of the symbiotic origin of the cell nucleus was introduced by Boveri (1888).

The beginning of the 20th century found the theory of horizontal exchange of biological information fully developed. Following detailed studies of chloroplasts, Merezhkowsky introduced the term symbiogenesis (1905, 1910, 1920). Famintsyn, who discovered the symbiosis among algae and radiolarians, believed that chloroplasts have a symbiotic origin and comparing physiology of bacteria and chloroplasts found interesting similarities. He hypothesized that chloroplasts (microbes) were in past phagocytosed as food, and for some unknown reasons were not digested but survived and adapted inside the cells of their new hosts (for review see Sapp 1994). They survived inside the cells for millions of years with the intact ability of independent reproduction.

Around the same time came the studies of French biologist Portier, who believed that mitochondria were also originally symbiotic bacteria (1918). However, his studies were strongly rejected. Wallin studied the same problem and pushed this hypothesis even further when he hypothesized that all types of symbioses played an extremely important role in evolution (1923,1927).

Only a year later the well-known transformation experiments using various mutants of *Streptococcus pneumoniae* differing in some properties occurred (Griffith, 1928). These experiments were the first proof of direct changes of hereditary characteristics of microbe by dead bacterial cells. Possible pathways of the horizontal transfer are summarized in Figure 1.

However, to characterize the substance causing this transformation was a problem. It was only in 1944 that Avery and his group showed that microorganisms are able to adopt foreign genetic material into their genome and that the responsible substance is DNA (Avery et al., 1944). In fact, this observation demonstrated that DNA is a molecule that encodes genetic instructions. Therefore, we can consider 1994 as the year when molecular genetics was born.

The work of Margulis (1981) can be considered to be the final pinnacle of theories about symbiosis. Her theories about the symbiotic origins of the nucleus led to the strong interest in studies of horizontal interspecies transfer of genetic material. Her work was followed up with studies showing that without viruses, both plants and living organisms would have evolved very slowly if at all and that the evolution of one organism depends on new "ideas" coming from other organisms (Anderson, 1970). Later it was found that the nucleic acid of chloroplasts and mitochondria are closer to that of bacteria than to the nucleic acids of eukaryotes. It is clear that the theory of symbiosis of two or more unrelated species with subsequent exchange of genetic information was more than 100 years older than the notion of mutuality of living organisms, which is now covered by the term, "mutualism" (Reed and Jones, 1977). Multicellular organisms did not evolve in splendid isolation from others organisms. It was not evolution, but co-evolution which formatted all living species. The horizontal transfer of genetic information substantially contributed to the evolutionary pressures and achievements, resulting in hierarchization of individual strata, structures and parts of the highly complex living structures of our planet. Across animal kingdom, numerous metabolic and defensive adaptations represent direct consequence of symbiotic association (Salem et al., 2015).

An endosymbiotic theory of the origin of organelles is now well accepted as a part of the concept of the lateral transfer of genetic information. Endosymbiosis, a coexistence of two or more taxonomically different organisms, forms only a part of the wide range of interspecies exchange of genetic information. From this point of view we can consider the incorporation of the whole genes or genomic sequences to be developmentally positive.

Eukaryotes have more genomes

Almost all living organisms with cells having nucleus (Eukaryota) have at least two closely cooperating genomes – one is the DNA from the nucleus, and the second is the mitochodrial DNA. Nuclear DNA is inherited both from mother and father; mitochondrial genes are transferred only from the mother. The integration of mitochondria to the cells is so strong, that nobody managed to cultivate them *in vitro*. However, they reproduce independently from nuclear DNA.

Plants and algae possess a third genome originating in chloroplasts. It is possible that during evolution, these organisms incorporated these organelles into the cells already containing mitochondria (with their DNA). Corals and some protists can

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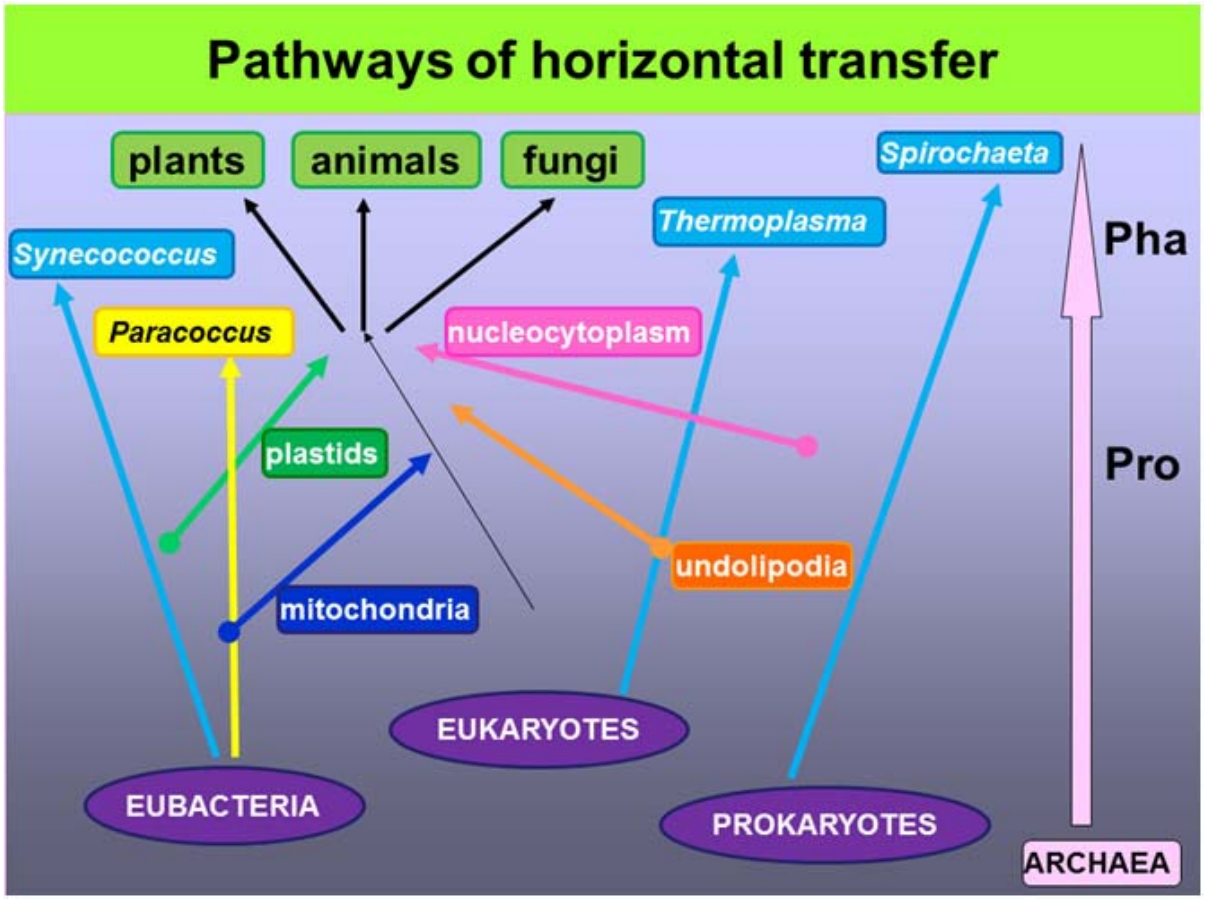

Figure 1. Pathways of horizontal transfer. Pha – phanerozoicum. Pro - proterozoicum

serve as exemplars of accepting new genomes. The flagellate *Mixotricha paradoxa* can serve as an example. It contains several bacteria, on the surface are additional adhered bacteria *Treponema* (Cleveland and Grimstone, 1964).

Individual ways of lateral transfer

Transfer of genes from organelles into the nucleus occurred during the evolution of eukaryotic genome. Some of these originally mitochondrial genes are now active in regulation and are under the supervision of nuclear DNA. As an example, we can use an enzyme malate dehydrogenase. It has prokaryotic character and its synthesis is regulated by nucleus. This horizontal transfer of genetic information across the interspecies barrier occurs either via merging of whole genomes of two or more organisms, or by insertions of significant regions of the genome (such as bacterial "islands of pathogenicity"). The transfer of individual genes or only their fragments is also possible. In this case, phages, plasmids and other mobile genetic elements such as transposons and retrotransposons and possibly even prions are involved (Lee, 1996, Hacker and Carniel, 2001, Schmidt and Hensel, 2004, Prusiner, 1994, Daubin et al., 2003).

Prokaryotes

Bacteria can be considered to have acted as genetic engineers for hundreds of millions of years. They successfully combine individual genes, genomic cassettes or whole genomes. Sex and infection are, for these highly promiscuous organisms, the same – movement and exchange of genetic information. When endangered, they merge without any immunological recognition and even unrelated bacterial species will exchange some of their genes. Horizontal gene transfer represents a universal mean for fast reorganization of the genome and an easy way to gain new characteristics, new reactions, and more adaptability to changing environmental conditions. It confers more rapid adaptive radiation, and on the other hand also increased pathogenicity and virulence or new resistance.

The fast spreading drug resistance can serve as an example. Resistant bacteria are living in the human gastrointestinal tract and in some antibiotic-treated patients, the surviving bacteria are released, and subsequently transfer the newly acquired resistance genes to bacteria of the same or different species. American statistics show that at the end of 1990, around 80% of the American population had tetracycline-resistant bacteria in their gastrointestional tract (Chopra and Roberts, 2001). Twenty years ago, the percentage was only 30. If resistance occurs only via mutations, the speed of this spread would be impossible.

Some studies suggested that over 90% of all genetic material in a given bacteria originated from other species. Daubin et al. (2003) found that in -proteobacteria, only 200 genes out of a total of 7,000 can be found in all these bacteria, which means that more than 97% arrived by horizontal transfer. It is therefore difficult to differentiate among individual bacterial species, making necessary diagnosis problematic in terms of accuracy. Horizontally transferred genes are characterized by codon usage close to that of genes implicated in virulence and antibiotic resistance, implying that the may be functional.

The total amount of horizontally transferred DNA differs mostly due to the size of genome. Mycoplasma with very limited genome has no such genes, 3% in *Mycobacterium tuberculosis*, or 15% in some strains of *Escherichia coli*.

With open access to the data of the complete genome sequences, more and more studies are focused on genome-based biology. Detailed studies showed that the majority of genes from complete genomes of *Archea* resemble counterparts among *Eubacteria* and not *Eukaryota* (Doolittle, 1998). A statistical procedure for predicting whether genes of a complete genome has been acquired by the horizontal transfer of genetic information was used in 17 bacterial complete genomes and 7 archeal ones. The results showed that the percentage of horizontally transferred genes varied from 1.5% to 14.5% (Garcia-Vallvé et al., 2000).

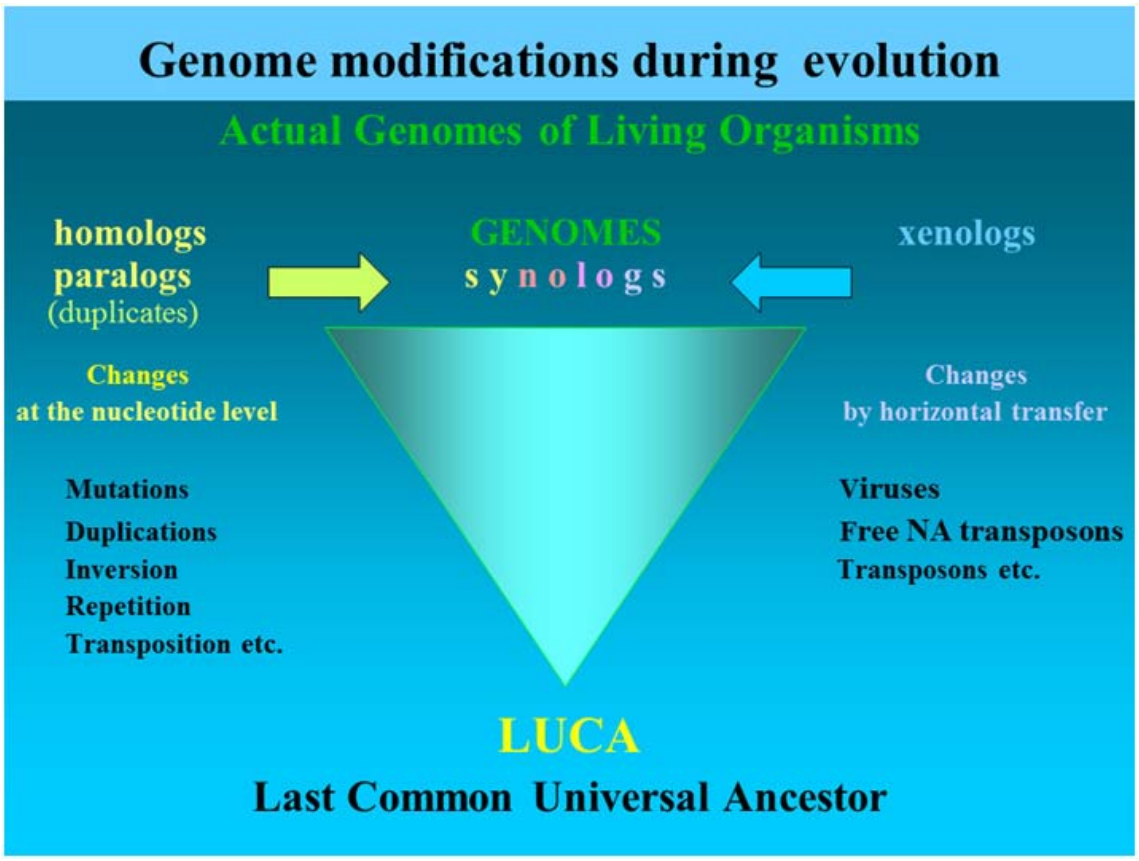

Figure 2. Genome modifications during evolution. NA - nucleic acid

Bacterial genomes

It is generally accepted that all past and current bacterial genomes had one common ancestor, so called Last Universal Common Ancestor (LUCA) (Woese 1998, Doolittle, 2000). Most evolutionary biologists and microbiologists believe that bacterial genomes developed via doubling of very small genomes of ancestors. However, the studies published by Nilsson's group (Nilsson et al., 2005) suggested exactly the opposite – they showed that bacteria with genome less than 2 Mb developed from bacteria with much bigger genome. This type of reductive evolution is characteristic particularly for current endosymbionts of eukaryotic organisms. These organisms lost over 80% of the original DNA and now lack even genes necessary for survival outside the host. Genome modification during evolution is summarized in Figure 2.

The loss of non-functional, and therefore unimportant genes, is not simple. In humans, we still keep hundreds of pseudogenes for olfactory receptors, some of which can be traced to the time of first tetrapods. Also, bacterial pseudogenes are kept for some time, but in the end, they are eliminated via mutations. The elimination processes are so strong that sometimes even the functional genes disappear. Pathogenic *Salmonella typhimurium* contains all genes that we can find in bacterial endosymbionts of *Buchnera* spp., *Blochmannia* spp., and *Wigglesworthia*, spp., suggesting that all these bacteria might have a common ancestor.

Eukaryotes

Multicellular organisms did not evolve alone, but were formatted by coevolution. For those processes, horizontal information transition might be the optimal tool. Cells of eukaryotic organisms are able to incorporate foreign genes into their genomes. Almost all multicellular organisms contain proviruses in their chromosomes. These proviruses are either full viral genomes or their parts, most probably obtained via infection. In most cases, these foreign parts are inactivated by mutations or neutralized by methylation. Retroviruses often serve as a vector of this transfer of foreign genes. The same processes are now employed in genomic manipulations (Youngsuk et al., 2011). In addition, theoretical studies indicate that selfish replicons (genetic parasites) inevitably emerge in any sufficiently complex evolving ensemble of replicators which means that the key signature of the greater virus world is genetic, i.e., various degrees of reliance on the information processing systems of the host (Koonin and Dolja, 2014).

Entomologists were for decades puzzled by the question of why so many different species of insects exist. Insects in general and beetles in particular managed to incorporate the bacterial DNA more than any other species (Weinert et al., 2007). The whole range of bacterial species exists in almost all insect tissues and they are often transferred to new generation by vertical transfer, i.e., via eggs. An interesting study showed that eleven separate genes in the genome of a beetle *Callosobruchus chinensis* originated in bacterium *Wolbachia* (Nikoh et al., 2008). *Wolbachia* represents maternally inherited endosymbiotic proteobacteria found in both terrestrial arthropods and filarial nematodes (Salunkhe et al., 2014).

These studies were the direct proof of the horizontal transfer of genes from a prokaryotic symbiont into the nuclear DNA of the eukaryotic host. One can easily imagine that the horizontal transport of genetic material helped to achieve such overwhelming variability of insects. A study of the taxon showed that at least some members of this taxon have evolved more recently than previously believed. It seems that mitochondrial symbiosis predates all *Archezoa* and maybe even all presently known eukaryotes (Keeling, 1998).

Recombination Activation Genes (RAGs) and the immune system

The human genome has at most 30,000 genes, and yet generates millions of different antibodies by cutting and recombining a few hundred genes. RAG-1 and RAG-2 genes are transposons coding for recombination of genomic segments of antigen-binding sites on the immunoglobulin molecule during VDJ recombination, and subsequently allowing the existence of this extreme antibody diversity (Sakano et al., 1979, Agrawal et al., 1998).

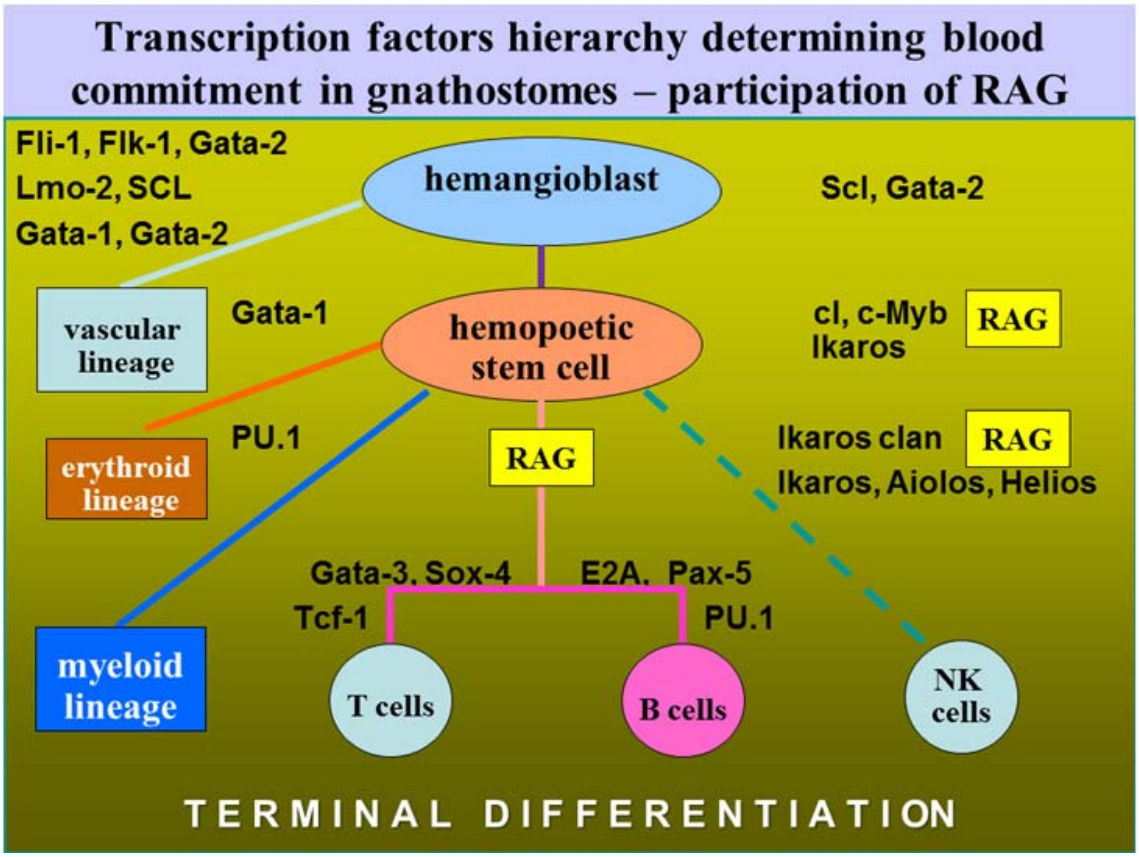

Figure 3. Transcription factors hierarchy determining blood commitment in gnathostomes – participation of RAG.

RAG-1 and RAG-2 genes are essential to the generation of mature T and B lymphocytes, and were most probably incorporated into genomes of ancient jawed vertebrates (*Gnathostomata)* as bacterial transposons. Several similarities with other transposons suggest horizontal transfer - transcription of DNA is the same as that in other mobile elements, and similar integrations occur in the case of HIV virus.

The most commonly suggested ways of horizontal transfer are infectious processes. It is possible that it first occurred in *Cephalochordata* (small eel-like animals), which possess a homolog of RAG-1 activator. Similarly, there are no RAG genes in *Agnatha* (a class of jawless fish), therefore there are no immunoglobulin receptors and no antibodies. Thus, it is assumed that RAGs appeared after the agnathan/gnathostome split. However, recent studies found the core sequence of Rag-1 in the echinoderm *Strongylocentrotus purpuratus* (Fugman et al., 2006). In these theories, RAG-1 appeared in the very early ancestors of all deuterostomia and was later lost in agnathans or that it invaded the genome several times (Flajnik and Masahara, 2010).

The sources of RAG might be bacteria which managed to enter the germinal lymphocytoid lineage where they adapted to the intracellular life. However, it is unknown as to why RAG-1/2 are found only in lymphocytes and not in other cell types. One explanation might be the fact the recombination of gene segments in antigen receptors represents such deep intervention into the genome that is was strictly limited to the lymphocyte lineage (Hansen et al., 2000) The high number of errors, resulting in blood cell cancers, is a result of such complicated processes. Figure 3 summarizes the transcription factors hierarchy determining blood commitment in gnathostomes and participation of RAG.

The resources for horizontal transport

Prokaryotes and eukaryotes not only accept and incorporate genes, but also secrete their own nucleic acids. Free molecules of nucleic acids, their parts and/or individual nucleotides can be found in relatively high concentrations in an aquatic environment. The source is mostly plankton. This DNA can, under optimal conditions, survive hours and sometimes even days (Bjorkman and Karl, 2001).

It is often neglected that for all organisms, nucleic acids form a significant part of food. It has been repeatedly demonstrated that enterocytes do not synthesize their nucleotides *de novo* (or only in very limited amounts), but utilize them from the food (Grimble, 1994). A single mitosis requires around 10^9 nucleotides, with correspondingly huge numbers required for daily renewal of cells in guts, lung, skin or blood.

The gastrointestinal tract contains enzymes that are able to degrade nutritional DNA, but most probably some parts of this DNA escape and are transferred via the intestinal barrier into the internal milieu, where they are internalized by cells. Experiments evaluating mice fed only by DNA showed that there is a shortage of these DNA-degrading enzymes. Segments of this nutritional DNA were found not only in cells of the gastrointestinal tract, but also in peripheral blood cells and cells in the spleen and liver.

Conclusion

Biologists have recognized for a long time that new characteristics have to be stabilized by natural selection. The same is true for the lateral gene transport. The species with extreme properties or with highly specialized attributes will eventually die. Lateral transfer of genetic information seems to play an important role in some evolutionary processes with often serious consequences. The question whether the currently living bacterial parasites such as *Salmonella, Shigella*, or *Franciscella* can become carriers of new genetic information remains. Another question might be the possibility of the appearance of new characteristics, organs or functions in current plants and organisms, similarly as it happened during Cambrian adaptation. Lateral (horizontal) transfer of genetic information confers more rapid adaptive radiation, and on the other hand also increases pathogenicity and virulence or new resistance.

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