

Cell Evolution in Fast Motion

How were plant cells, and thus higher forms of life on Earth, able to evolve from bacteria? **RALPH BOCK**, Director at the **MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY** in Golm, has been exploring this question for many years. The object of his research: tobacco cells. Like all plant cells, they have DNA not only in the nucleus, but also in two other organelles: the chloroplasts and mitochondria. In the course of evolution, genes migrated from the hereditary material of these organelles into the nucleus. Using new technologies and selection procedures, Bock and his team succeeded in reproducing this process in the laboratory and examining it under experimental conditions.

ILLUSTRATION: JOCHEN STURMANN

Corals glow in a huge variety of colors. Their cells are inhabited by algae that not only give them their attractive appearance, but also fulfill an important function. They carry out photosynthesis and are thus capable of converting light into chemical energy, and carbon dioxide into sugar. This process also releases oxygen – the prerequisite for all life on our planet. This phenomenon has one crucial advantage for the coral: it does not have to nourish itself exclusively from the microplankton it filters out of the sea water, but can also benefit from the products generated by the photosynthesis carried out by the algae in light-filled surface waters.

Nitrogen-fixing bacteria inhabit the root nodules of legumes – that is, plants that bear podded fruits, such as beans. Therefore, the bacterium *Rhizobium japonicum* lives in symbiosis with the soybean, for example. As part of this close working relationship, the plant provides carbohydrates and other organic compounds, while the bacterium is able to bind atmospheric nitrogen from the soil, which the plant can use to form amino acids in various ways.

So humans are not the only organisms that form relationships. Complicated forms of contact arise between individual cells, bacteria, animals and plants. In nature, they can be loose or close, competitive or symbiotic, or to the advantage or the detriment of a given species. The transitions are often fluid and change in the course of evolution.

For Russian nature researcher Constantin Sergeevich Merezhkowsky, such symbiotic relationships provided an initial indication that new life forms can emerge from combinations of individual organisms. Merezhkowsky published his first theoretical study in 1905, which is still viewed as the essential text on the theory of endosymbiosis today.

According to this theory, mitochondria and chloroplasts stem from

organisms that were originally independent and that were probably consumed by a prokaryotic precursor cell over a billion years ago but not digested. Like amoebas, which envelop their food and ultimately absorb it, the cells engulfed these organisms (free-living bacteria). The membrane of the host cell invaginated, the bacterium was completely assimilated into the interior of the host cell and then mercilessly enslaved: it lost its independence, became the mitochondrion and served its host cell thereafter as a powerhouse for the generation of energy. This cell type with two genomes – in the nucleus and in the mitochondria – is still found in all animals and fungi today.

CYANOBACTERIA BECOME CHLOROPLASTS

In addition to the mitochondria, plant cells have another type of DNA-containing organelle: the “green” chloroplasts. The process described above was repeated during the emergence of the plant cell. In this case, however, a cyanobacterium, a so-called blue-green alga, was ingested. This organism was already capable of photosynthesis – in other words, it could convert light energy into chemical energy and carbon dioxide into sugar, releasing oxygen in the process. Therefore, both the chloroplasts and mitochondria are surrounded by two membranes: the external membrane originates from the host cell, and the internal one from the incorporated bacterium. Today, Merezhkowsky’s theories on the origin of these cell organelles constitute a major component of the modern (synthetic) theory of biological evolution.

However, many questions remain open. For example, how were the incorporated bacteria gradually transformed into cell organelles in the course of evolution, and how were the participating genomes restructured? All that is definitely known to date is that an enormous volume of

bacterial genes was transferred from the hereditary material of the incorporated bacteria into the nuclear genome of the host cell. Today, the organellar genomes contain only a few dozen genes, although the bacteria from which they originated probably contained at least a few thousand genes. Sequence similarities between nuclear genes and genes from cyanobacteria provide indirect indications of this kind of gene transfer from the mitochondria and chloroplast genomes to the nuclear genome. Cases involving genes that were clearly transferred only a relatively short time ago also exist. In some plant species, such genes are still found in the organelles, while in other species, they can already be found in the nuclear genome.

How can a gene from a chloroplast or mitochondrion, which are bounded by a double membrane, enter the nuclear genome? Because the transfer of thousands of genes from the organelles to the nucleus clearly took place over vast evolutionary periods and, as a result, no one has ever been able to observe such an event, this question has hitherto eluded all access by experiment. It is only as a result of the development of new technologies that, through genetic engineering, enable the alteration of the chloroplast genomes of higher plant species that we have been able to reproduce in recent years – practically in “fast motion” – important steps in this evolutionary process in the laboratory, and to analyze the molecular basis of the transfer of genes between organellar genomes and nuclear genomes.

Using a simple experimental ruse, we succeeded in reconstructing this gene transfer at the Max Planck Institute of Molecular Plant Physiology in Golm: we altered the chloroplast genomes of tobacco plants through the introduction of two additional genes using gene technology. While one of the two genes, known as *aadA*, is tailor-made for the chloroplast genome

due to its bacterial genetic structure, we engineered the second gene, *nptII*, for the nucleus (eukaryotic gene structure). The *aadA* gene provides resistance to the antibiotic spectinomycin, and *nptII* provides resistance to the antibiotic kanamycin.

This enabled us to make a simple selection of plants in which the gene transfer to the nucleus had taken place: because the *nptII* is not active in the chloroplasts, plants with transgenic chloroplast genomes are resis-

gene transfer from the chloroplasts to the cell nucleus had taken place: the *nptII* gene had entered the nucleus in approximately one out of five million cells. This finding altered our understanding of genetic homogeneity within a species and within an organism. When one considers, for example, that a tobacco leaf comprises significantly more than five million cells, it becomes clear that the cells in one and the same leaf on a plant are not necessarily geneti-

cannot be read and translated into messenger RNA automatically. The same applies to genes transferred in the opposite direction. They lack the specific promoter, a sequence located upstream of the gene that facilitates its transcription, and the specific terminator, which guarantees the stability of the messenger RNA formed.

In the above-described laboratory experiment, we were able to get around this problem by equipping the *nptII* gene with eukaryotic ex-

pression signals, that is, a corresponding promoter and terminator, which could be active immediately after the transfer. However, the question remained as to how this process occurred in nature and how a functional nuclear gene can emerge from a former chloroplast one.

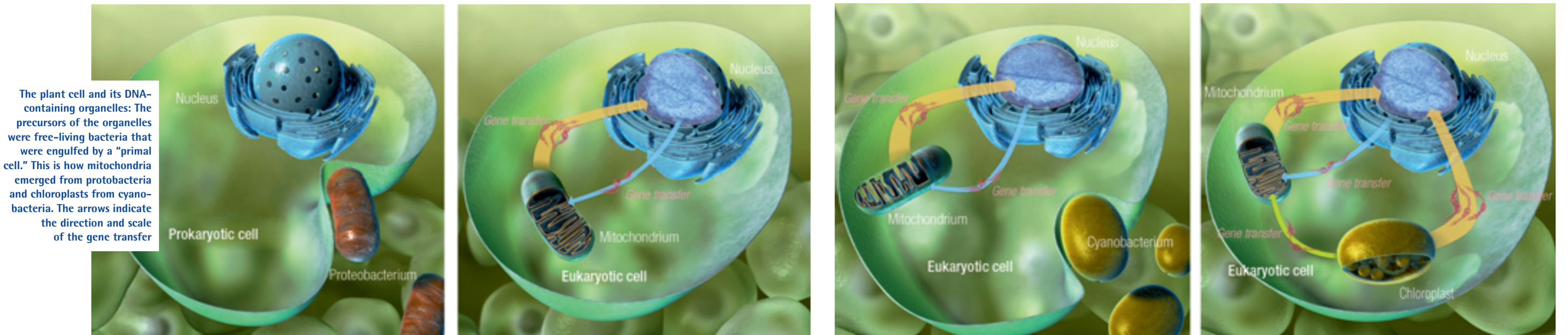
The answer was provided by plant lines that had transferred a big piece of chloroplast DNA to the nucleus, on which not only the kanamycin-resistance gene *nptII*, but also the neighboring spectinomycin-resistance gene *aadA* were located. The *aadA* gene provided us with exactly the right test object: as a prokaryotic gene (with a chloroplast promoter and terminator), it was not active in the nucleus. As a result, the gene transfer plants with

the *aadA* gene in the nucleus reacted sensitively to the antibiotic spectinomycin. In a large-scale selection experiment, we then examined whether and how this inactive *aadA* gene can become functional in the nucleus. To this end, cells were exposed to culture medium containing spectinomycin. In this way, we actually obtained plants in which the *aadA* gene in the nucleus had become active.

The next step involved clarifying the molecular mechanisms that had

mechanism, although the molecular mechanisms used to halt the reading process in the nucleus are completely different than those in the chloroplast. We explain this in terms of the frequent presence of the nucleotide bases adenine (A) and thymine (T), which are frequently found in both chloroplast and nuclear terminators.

These experiments provided us with initial insights into the molecular processes that have shaped the genomes of animal and plant cells lapse, and of explaining the mechanisms on which they are based. In our current experiments, we are working on reconstructing the horizontal gene transfer between organisms and plant species in the laboratory. Critics of genetically modified foods fear that genes introduced into plants could be transmitted to other unrelated organisms, for example to micro-organisms or other plant species. This process, known as horizontal gene transfer, has been the object



The plant cell and its DNA-containing organelles: The precursors of the organelles were free-living bacteria that were engulfed by a "primal cell." This is how mitochondria emerged from proteobacteria and chloroplasts from cyanobacteria. The arrows indicate the direction and scale of the gene transfer

tant to the antibiotic spectinomycin, but react sensitively to kanamycin. When the cells of these plants in a tissue culture system undergo selection for kanamycin resistance, only those cells that have transferred the *nptII* gene from the chloroplast genome into the nuclear genome survive.

Genetic and molecular tests can then be used to confirm successful gene transfer: the kanamycin resistance was inherited in accordance with Mendelian rules (while chloroplast genes, and thus spectinomycin resistance, are inherited only maternally), and we were able to demonstrate the presence of the DNA of the *nptII* gene in the nuclear genome. However, we were surprised by the frequency with which this kind of

cally identical. They can actually differ in terms of the pattern of the organellar DNA sequences transferred to the nuclear genome.

Another interesting consequence of this process arises from the random insertion of transferred organellar genes into the nuclear genome. It is conceivable that insertion into a gene of the nuclear genome will occur and thus destroy the latter. Therefore, the transfer of organellar DNA to the nucleus can also contribute to the spontaneous appearance of mutations (so-called "somatic mutations").

However, the successful transfer of a gene from the chloroplasts to the nucleus does not result directly in a new functional nuclear gene, as genes originating from the chloroplasts

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led to the activation of the *aadA* gene. It emerged here that very similar processes were involved in the gene activation in all eight selected plant lines. In all cases, the *aadA* gene succeeded, by means of relatively simple mutations (so-called deletions), in "capturing" the promoter of the neighboring nuclear gene and in using it for its own expression. Astonishingly, molecular restructuring of the latter was sufficient to make the nuclear gene capable of functioning.

We expected similar changes in the terminator area, but were unable to demonstrate them. Surprisingly, an analysis of the messenger RNA belonging to the *aadA* gene revealed that the chloroplast terminator could be used by the nucleus's reading

since their emergence more than a billion years ago. Using new gene technology methods and genetic selection processes, it has become possible to allow these processes to unfold in the laboratory over a period of just a few years. This opens up the exciting prospect of reproducing processes that normally take place over vast evolutionary periods in time-

of research for more than 20 years. Although gene transfer between bacteria occurs regularly in, for example, hospitals, wastewater treatment plants and septic tanks, little research has been carried out on horizontal gene transfer from plants. Genetic experiments are now expected to clarify whether and how often plant genes can overcome species barriers. ■

ILLUSTRATIONS: JOCHEN STUHRMANN



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PROF. DR. RALPH BOCK is Managing Director at the Max Planck Institute of Molecular Plant Physiology in Golm and Head of the Department of Organelle Biology, Biotechnology, and Molecular Ecophysiology. He supports the European Food Safety Authority in developing safety standards for the release of genetically modified plant species.



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BARBARA ABRELL studied botany at the Technical University Munich and wrote her thesis in limnology on the role of algae in hypertrophic oxidation ponds. She worked as a science editor for FOCUS online for several years. She has been employed at the Max Planck Society Press Office since 2007.