The plastid skeleton: a source of ideas in the nano range

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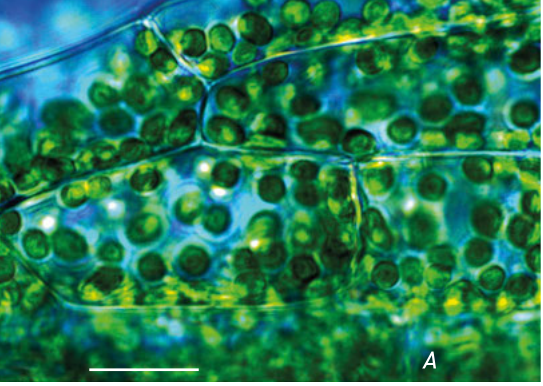
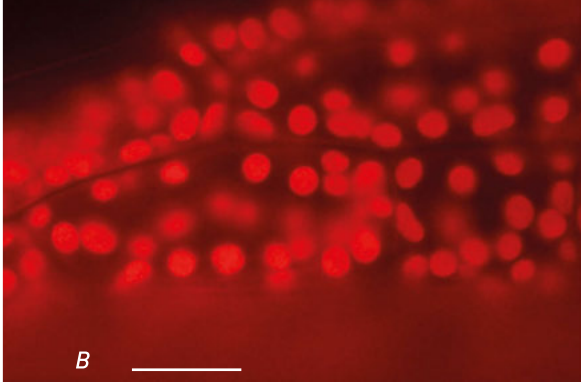
All life on earth relies on the conversion of solar energy into chemical energy through the process of photosynthesis, in which the greenhouse gas carbon dioxide (CO_2) is absorbed and oxygen (O_2) is released. The green parts of plants are capable of performing this reaction. This is where we find chlorophyll, the green pigment that captures sunlight. Chlorophyll and all components of the conversion process do not exist freely in the plant cells, but are located in certain "reaction spaces," the chloroplasts. These are part of what are called organelles, small reaction spaces within the cell that are separated from other cell components by a double membrane of lipids and proteins.

Plastids with skeleton

Chloroplasts are normally lens-shaped ⌐152. But they can also change their shape, that is, they can grow and divide. For a long time it was not known what causes these changes, what structure gives the organelles their shape, and what is responsible for changes in that shape. We biologists were able to demonstrate that the chloroplasts of a moss, the spreading earthmoss (*Physcomitrella patens*), contain five different so-called FtsZ proteins. When we mark these FtsZ proteins using genetic methods by attaching the bright-green fluorescing GFP protein, microscopic images reveal protein filaments and networks ⌐153. It is noticeable that each FtsZ protein is characterized by a pattern that is different from the other four. These patterns are reminiscent of the cell skeleton that occurs in the cytoplasm of every higher cell (eukaryotic cell), giving it its shape and helping it to change its form. For this reason we proposed the analogous term "plastid skeleton" for these FtsZ filaments in the chloroplasts. Microbiologists have been able to demonstrate that similar cell skeletons occur in bacteria, determining their shape and triggering division. Here too, an FtsZ protein is involved. When this is mutated in bacteria, they take on the shape of a thread at certain temperatures. This is also where the abbreviation FtsZ comes from: filamentous temperature-sensitive mutant Z. This finding is particularly exciting from the point of view of evolution, because the chloroplasts of plants evolved from bacteria about one and a half billion years ago. We can therefore surmise that the FtsZ molecules of bacteria are similar to those of chloroplasts not only in their composition and sequence but also in their function.

In this research project, biologists from Freiburg University and engineers from Stuttgart University have got together in order to uncover the secrets of the plastid skeleton in mosses. This is very challenging, because the structures investigated





152 *Cutout from a small leaf of the spreading earthmoss (*Physcomitrella patens*). Light microscopy using a Zeiss Axioplan2 microscope shows the green-colored chloroplasts. (B) The same cutout using fluorescence microscopy shows the red inherent fluorescence of the chlorophyll. Scale bar: 20 µm.*

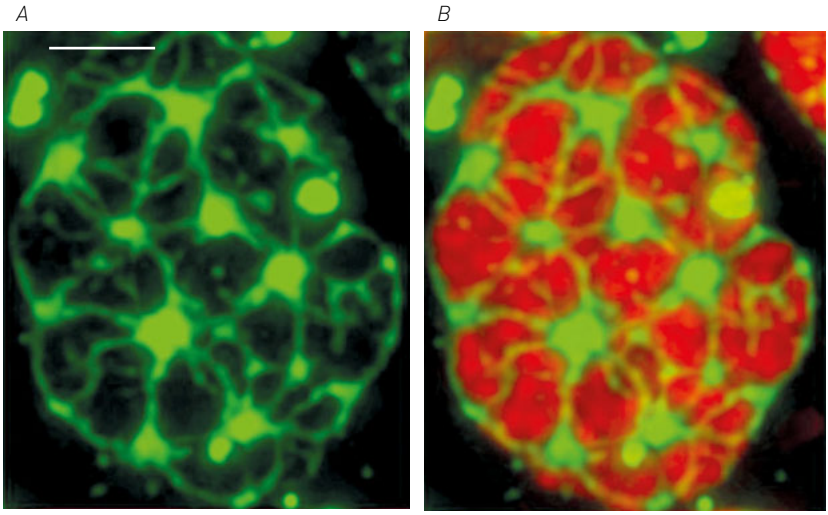
are minute: the moss plants themselves measure only a few millimeters, their chloroplasts have a diameter of just a few micrometers, and the FtsZ protein filaments have a thickness of a few nanometers. To make these visible, high-resolution microscopes are needed. We use confocal laser scanning microscopes for this purpose, special light microscopes that scan the structures with laser beams of certain wavelengths and then put together the individual light points to create images **153**. Because these images are produced from living cells in which the molecules are constantly moving, no two images are the same. For this reason it is necessary to make a great number of different images in order to obtain a good overview of all possible structures of the plastid skeleton. Since all of these images are electronic, the tests create a huge amount of data, which can be automatically processed in computers. For this, special methods are developed with which it is possible to describe the recorded structures of the individual protein networks with mathematical equations, thereby making them comparable.

From image to model

In microscopy, 2D layer images are created from 3D structures, a process known in medical imaging. Depending on the resolution, the amount of data, and the microscope, the distance between the individual images can vary. In order to actually produce 3D images of individual objects, a 3D geometry has to be created from the 2D images. The smaller the distance between the image slices, the more accurate the models. When the distance becomes too great, there is too much uncertainty about the space in between, and assumptions have to be made. This can lead to errors.

Once the models have been completed, they must then be converted into computer models. For this, a grid network has to be created. We can imagine how such a grid network functions if we compare it with Lego blocks: if we only have very big Lego blocks—analogous to a coarse grid network—we can only build a coarse structure, which can only approximately replicate a complicated object. If we choose smaller Lego blocks—or a finer

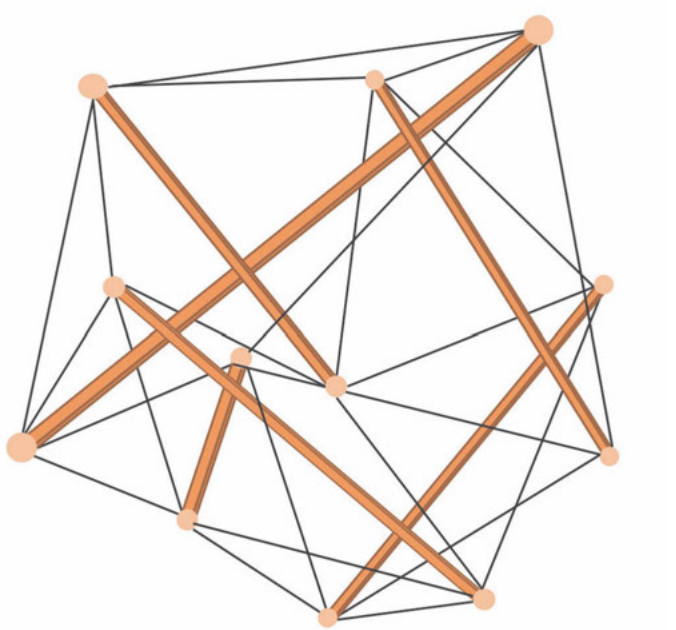
153 *(A) A single chloroplast under the Leica TCS 8T-WS confocal microscope. An FtsZ2-1 network shown in green. (B) The same image; here the chlorophyll is also shown in bright red. Scale bar: 2 µm.*



grid network—we can replicate the structure much better.

However, such a detailed replication also involves a large amount of data. After all, the geometry and position of each individual Lego block has to be determined. If we now want not only to reconstruct the geometric variables of the model but also to obtain information about how its function relates to its structure—for example, if we want to carry out a simulation of its mechanical strength—then we need to solve a set of mathematical equations for each Lego component. A very detailed model obviously increases the precision of the information/simulation, but at the cost of the general computing time. For very high resolutions, we need to use one of the supercomputers that have been purchased for several million euros by certain computer centers, such as the High Performance Computing Center Stuttgart (HLRS). This means that, taking practical considerations into account, a good balance has to be struck between computing time, resources, and precision.

In this way it is possible to achieve a mathematically exact description of the different FtsZ networks of the plastid skeleton and to differentiate them. Initial results show that the five FtsZ networks differ from each other; one hypothesis is that, together, they form what is known as a tensegrity structure **154**. Such a structure consisting of bars and tensioned cables was initially invented by two architects and refers to a stable construction that uses only a minimal amount of material.



154 *Model of a tensegrity structure, a strut system in which the struts are connected with each other by tensile elements (such as cables). The tensile stress in the elastic parts (black) stabilizes the system and makes it dynamic.*

In our ongoing work we want to check the hypothesis that the plastid skeleton is a tensegrity structure, and investigate how the plastid skeleton changes when exposed to loads, during growth, and in the division of chloroplasts. To this end, we have already carried out initial tests using a scanning force microscope, which we can use to mechanically scan the chloroplast surface and measure even minute forces at the nanometer scale. The objective of our project is to shed light on the dynamics and mechanics of the plastid skeleton, to describe it mathematically, and to use this information to derive general principles that could be used at much larger dimensions (i.e., in engineering and architecture).

In order to achieve this objective, we will take the path of "reverse biomimetics." This means that the detailed analysis of the plastid skeleton will be used to establish hypotheses and models; furthermore, it is intended to produce functioning prototypes (demonstrators). This transfer from a very small scale to a much larger one—from nanometer to centimeter—will lead to new findings and hypotheses, which in turn will be examined using specific genetic changes of the moss. The knowledge gained in this process will then lead to improved models and demonstrators. In this way, not only can architects learn from nature, but biologists can learn from engineers and architects. That is the particular scientific attraction of this project.