Can the chloroplast division machinery be evolutionarily dated back to bacteria?

The chloroplast, known to have originated around a billion years ago, is now one of the most recognizable characteristics of the plant cell. After the engulfment of the ancient cyanobacterium by the eukaryotic host cell, the former free-living organism (bacterium) evolved into a chloroplast in the plant cell through endosymbiosis (Fig. 1). As a result, even now, the chloroplast proliferates by binary fission similar to that in bacteria. The chloroplast division machinery is a hybrid structure comprising inner and outer elements. The inner space of the chloroplast (stroma) is topologically equivalent to the bacterial cytosol. The bacterial division ring, the “Z ring,” which is a key player in the constriction and subsequent splitting of a bacterial cell, has also been conserved. It mainly consists of a FtsZ tubulin homolog and functions as an inner part of the chloroplast division machinery (Fig. 1).

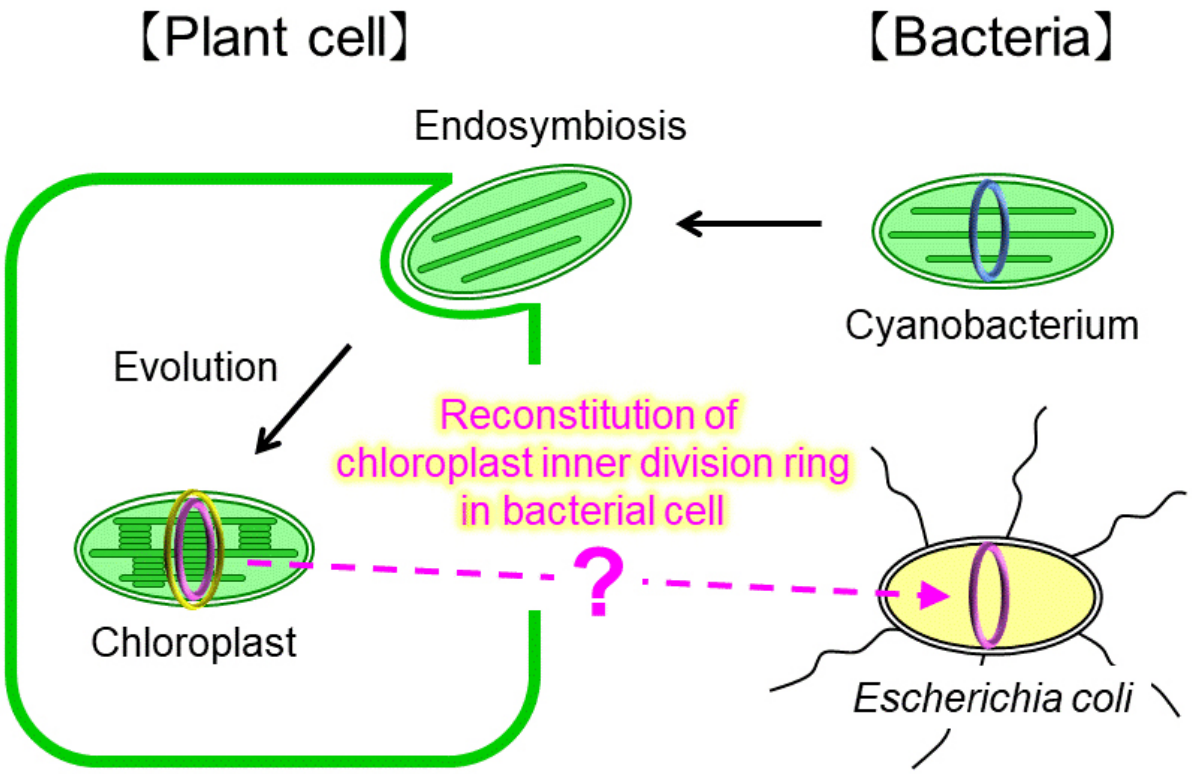

Fig. 1. Chloroplast evolution from bacterium and their division rings. The chloroplast has inherited its inner division ring (Z ring) from bacteria to some extent. The purpose of our study is to reconstitute the chloroplast Z ring in bacteria.

Reconstitution of the chloroplast Z ring in a bacterial cell is of great interest from the view of the evolutionary stability of the Z ring-centered division machinery. In addition, it could serve as a tool to study the behavior of each molecule that contributes to the chloroplast Z ring dynamics. This simple system could also support chloroplast research in plant cells, where many related components act together in a complex manner. It has been previously reported that when expressed, the constituent molecules of the chloroplast Z ring (the FtsZ protein) in the bacterial cell polymerize and form long filaments. Accordingly, it is no easy task to attempt to reconstitute “the ring” of chloroplast FtsZ, and there were some issues that required to be resolved.

As published in the Scientific Reports, we have addressed these issues using *Escherichia coli* as a model bacterium. We successfully developed a heterologous reconstitution system for the chloroplast Z ring. The most important factor was the tethering of the FtsZ filaments to the membrane. Chloroplast FtsZ does not have a domain for membrane anchoring and cannot attach to the membrane in itself. In this context, our first success of the Z ring-like structure formation was achieved by artificial tethering using a membrane targeting sequence (a derivative of a membrane anchoring protein from *E. coli*) added to the chloroplast FtsZ. In the second case, we challenged the co-production of chloroplast proteins FtsZ and ARC6, a putative tethering partner of FtsZ in chloroplasts. We found that ARC6 strongly promotes “the ring” formation of FtsZ in bacterial cell. Thus, on reconstituting the Z ring of chloroplast FtsZ in this system, we have demonstrated that ARC6 is the genuine tethering protein of chloroplast FtsZ to the membrane.

In contrast to ARC6, another chloroplast protein ARC3 inhibited filament and ring formation of chloroplast FtsZ in the bacterial cells. This is consistent with previously published studies, further supporting the value of our bacterial reconstitution system for chloroplast division research. Chloroplast division mobilizes many components, including the positive and negative regulators of Z ring formation as well as the constituent molecules of the Z ring. Many of these components employed in the inner division machinery could be applied to our novel bacterial system. Therefore, our study sheds new light on the perfect reconstitution of chloroplast inner division machinery in its progenitor bacterium, as if it were going evolutionarily back in time (Fig. 1).

Hiroki Irieda [1], ***Daisuke Shiomi*** [2]
[1]*Academic Assembly, Institute of Agriculture, Shinshu University, Japan*
[2]*Department of Life Science, College of Science, Rikkyo University, Japan*

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