

Origin of the chloroplast division mechanism

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Abstract

The chloroplast division mechanism is similar to bacterial division. Considering chloroplasts are from bacterial origin, engulfed bacteria evolved and adapted to the eukaryotic host-cell. Parts of the bacterial division components were retained and parts of the eukaryotic host-cell were integrated. The chloroplast division complex consists of four contractile rings, while the bacterial division is only comprised of one contractile ring. The first contractile ring to assemble is of bacterial origin; the FtsZ ring. Then the inner PD ring, outer PD ring and ARC5/DRP5B ring follow. The contractile rings need to be tethered to the membranes and are regulated by other proteins. The contractile rings on the inner envelope membrane are regulated by the proteins ARC6 and PARC6 and the outer envelope membrane is managed by the proteins PDV1 and PDV2. It is also very important that the contractile rings are placed on the correct division site of the chloroplast. This is directed by the proteins ARC3, minD, minE and MCD1. The membranes need to be separated for the final segregation. Proteins that are thought to be involved in this process are CLMP1 and CRL. Certain factors of the division mechanism are still not fully understood and need to be investigated.

Introduction

Chloroplasts are vital components that are found in plants and algae. Chloroplasts are primary plastids that are responsible for the photosynthetic conversion of carbon dioxide into carbohydrates by using light. Chloroplasts are derived from cyanobacteria through endosymbiosis. The engulfed bacterium was retained by a eukaryotic host-cell, instead of being digested. After the endosymbiosis, the bacteria evolved into chloroplasts and adapted to the host-cell. It gained a double membrane and lost the peptidoglycan cell wall. Similar to bacteria, chloroplasts replicate by binary fission. [1]

Chloroplasts have retained a lot of features of the cell division machinery of their prokaryotic ancestor. The division process of the bacteria also needed to evolve in order to adapt to the collaboration with the eukaryotic host-cell. Therefore, the chloroplasts transferred a few genes to the host nucleus. This means that the division components that work on the inner membrane need to be transported inside the organelle and components of the chloroplast cell division mechanism are both of cyanobacterial endosymbiont and eukaryotic host-cell origin. In the course of evolution, parts derived from cyanobacteria were retained and parts that are of eukaryotic host-cell origin were integrated. [2] Most of the proteins involved in chloroplast division have been identified by detecting homologs of bacterial cell-division proteins and by molecular genetic studies on the chloroplasts in *Arabidopsis Thaliana* and in the unicellular red alga *Cyanidioschyzon merolae*.

In this report the mechanism of chloroplast division will be discussed and the origin of components in the chloroplast cell dividing mechanism will also be described.

Division mechanism

The inner and outer envelope membranes of the chloroplast need to be constricted simultaneously at the division site during cell division. Two electron-dense ring structures at the division site have been detected and are called plastid-dividing (PD) rings [3]. One is located on the surface of the inner envelope membrane (inner PD ring) and the other on the surface of the outer envelope membrane (outer PD ring). The constriction of the envelope membranes is believed to be directed by four distinct contractile rings (shown in figure 1); two are inside the chloroplast and two outside. The internal rings are composed of the filamentous temperature-sensitive Z (FtsZ) ring and the inner PD ring and external rings are the accumulation and replication of chloroplasts 5 (ARC5)/Dynamamin-Related protein 5B (DRP5B) and the outer PD ring. [4, 5] Whilst the bacterial cell division machinery is only controlled by a single contractile ring: the FtsZ ring. [6]

The origin and functions of the components of the chloroplast mechanism are listed in table 1 below. All components will be individually discussed in the coming paragraphs and the functions will be described.

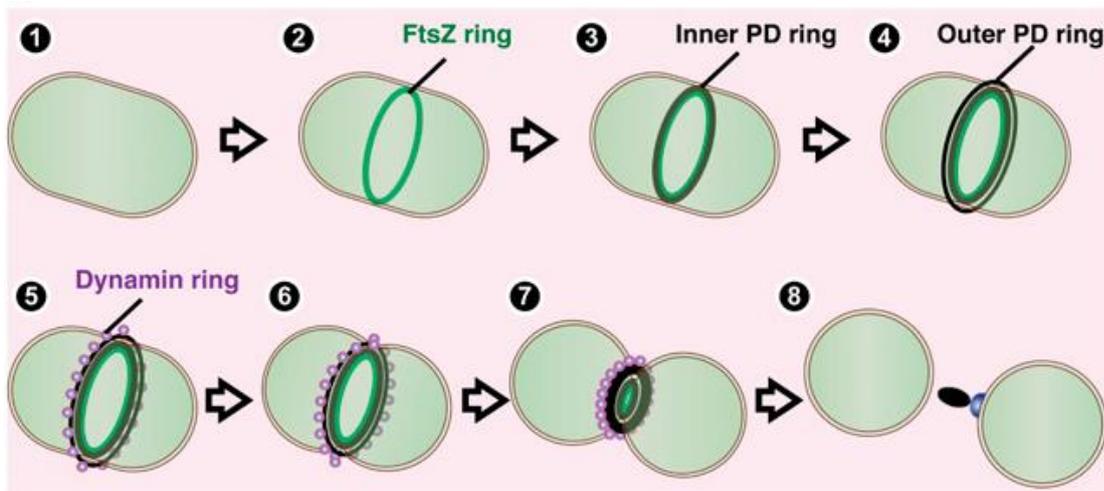


Figure 1: Assembly of the four contractile rings of the chloroplast division complex. [4]

Table 1: Components of the chloroplast division mechanism.

Component	Origin	Function
FtsZ1/FtsZ2	Cyanobacteria	The FtsZ ring is the first contractile ring to form in chloroplast cell division and is of bacterial origin. Bacterial FtsZ self-assembles into a ring structure that can constrict at the division site. The chloroplast division machinery contains two types of FtsZ proteins. FtsZ1 co-assembles with FtsZ2 into a ring structure to constrict the inner envelope membrane. FtsZ1 was created by gene duplication of the FtsZ2 protein after endosymbiosis and lacks the short C-terminal peptide. The C-terminal peptide is responsible of the interaction between FtsZ2 and ARC6.

Component	Origin	Function
Inner PD ring	Eukaryotic host-cell	The inner plastid dividing (PD) ring assembles after the FtsZ ring is formed. It was identified by electron microscopy and has a belt like structure with a thickness of approximately 5 nm. The composition of the inner PD ring has not yet been determined.
Outer PD ring	Eukaryotic host-cell	The outer PD ring is the third contractile ring to form on the outside of the envelope membrane. The protein plastid-dividing ring 1 (PDR1) was found in the red algae <i>C. merolae</i> . This protein is involved in the synthetization of the outer PD ring and is composed of a bundle of polyglucan filaments.
ARC5/DRP5B	Eukaryotic host-cell	ARC5/DRP5B is the final contractile ring that is formed. ARC5/DRP5B is related to the eukaryotic dynamin superfamily. In eukaryotic species, dynamins are GTPases that are involved in cytokinesis. ARC5/DRP5B is essential for constriction of the outer envelope membrane of the chloroplast.
ARC6	Cyanobacteria	ARC6 promotes FtsZ-ring assembly and tethers the ring to the inner envelope membrane. It also positions PDV2 at the division site.
PARC6	Cyanobacteria	PARC6 is a paralog of ARC6. It is involved in inhibiting FtsZ ring assembly at non-division site by interaction with ARC3. It also positions PDV1 at the division site.
PDV1/ PDV2	Eukaryotic host-cell	PDV1 and PDV2 help in the recruitment of DRP5B and promote the DRP5B contractile activity. It may be involved in the regulation of DRP5B GTPase activity.
ARC3	Eukaryotic host-cell	ARC3 is involved in the chloroplast min system and is a functional paralog of bacterial minC, although they are not similar in sequence. It inhibits the formation of the FtsZ ring at non division sites through direct interaction.
MinD	Cyanobacteria	MinD is part of the chloroplast min system. It is involved in managing the positioning site of the FtsZ ring and inhibiting from forming at non-division sites.
MinE	Cyanobacteria	MinE is part of the chloroplast min system. Helps regulate the location of the FtsZ ring.
MCD1	Eukaryotic host-cell	MCD1 is also involved in the placement of the FtsZ ring. It helps locating minD to inner envelope membrane.
CLMP1	Eukaryotic host-cell	Mutant analysis in <i>Arabidopsis thaliana</i> indicate that CLMP1 is required for chloroplast segregation. Chloroplasts lacking CLMP1 were clustered together after constriction and were held together by a thin membrane isthmus. The mechanism of chloroplast segregation remains unknown.

Component	Origin	Function
CRL	Cyanobacteria	CRL is also a protein thought to be involved in chloroplast segregation. Mutant CLR cells in <i>A. thaliana</i> presented few and enlarged chloroplasts and failed to segregate.

FtsZ ring

The first contractile ring to form in chloroplast division is the FtsZ ring and is of bacteria origin. It is the main component of the bacterial cell division machinery. It is an assembly of polymeric structures at the division site. FtsZ is a prokaryotic homolog of the eukaryotic tubulin protein family and is one of the main component of the eukaryotic cytoskeleton. It is composed of multiple distinct domains. An N-terminal domain is composed of the GTP-binding site and the lower site of the interface of the protofilament bond. The C-terminal domain contains the upper site of the interface and includes a T7/synergy loop. The amino acid sequence following the C-terminal is very diverse between species and acts as a flexible, unstructured linker and is of variable length. The final amino acids of the C-terminal is a highly conserved region and is the binding area of the FtsZ membrane-tethering proteins, such as Zip A and FtsA found in *Escherichia coli*. FtsZ protein monomers assemble into protofilaments in the presence of GTP by binding the N-terminal to C-terminal. The GTP is then positioned between the FtsZ monomers and behaves as a GTPase-activating protein. GTP hydrolysis can then be used to generate a constructive force that pulls in the membrane. [7, 8]

FtsZ that occurs in chloroplasts has evolved into two different proteins by gene duplication and differentiation; FtsZ1 and FtsZ2. FtsZ1 lacks the short C-terminal peptide that is present in FtsZ2 and the bacterial homolog. In bacteria, the C-terminal can interact with membrane proteins ZipA and FtsA that tethers the Z ring to the cytoplasmic membrane. These proteins are not found in eukaryotes or the ancestral cyanobacteria. The C-terminal of FtsZ2 can interact with the inner envelope membrane protein ARC6 and is thought to attach the FtsZ ring to the inner envelope membrane. Protein ZipN occurs in the cyanobacteria and is an ortholog of ARC6 and interacts directly with FtsZ. It has been found that it has similar properties of the bacterial FtsA. [9] [10] It has shown by immune fluorescence microscopy that FtsZ1 and FtsZ2 are both localized at the chloroplast midpoint formed in a co-assembled FtsZ ring. [11] Studies involving the moss *Physcomitrella patens* and *Arabidopsis Thaliana* showed that the absence of either FtsZ1 or FtsZ2 resulted in a disruptive chloroplast division pattern and having cells with fewer and larger chloroplast than normal cells. The evidence in these studies suggests that both FtsZ proteins are vital for chloroplast cell division. [12] [13] [14]

Inner PD ring

The inner PD ring is the second contractile ring that assembles during chloroplast division. This component is of eukaryotic host cell origin and has been integrated into the chloroplast division mechanism. It has been detected by electron microscopy in several species of algae and land plants as a 5 nm belt located at the stromal side of the inner envelope membrane. The composition of the inner PD ring has not yet been identified. [3] [15]

Outer PD ring

The formation of the outer PD ring is located on the chloroplast outer membrane. The outer PD ring is composed of filaments with a thickness of approximately 5 nm. The components of the PD ring were recently identified in the red algae *Cyanidioschyzon merolae*. It is composed of a bundle of polyglycan filaments. A protein called Plastid-Dividing Ring 1 (PDR1) was found by proteomic analysis that is related to the eukaryotic glycogenin protein. Glycogenin proteins in eukaryotic species can form a primer that acts as a substrate for glycogen synthase. It is indicated by electron microscopy analysis that the PDR1 protein forms a ring with carbohydrates at the division site. The protein moves from the cytoplasm to the chloroplast after the Z ring is formed, stays there during constriction and then returns back to the cytoplasm. Decreased chloroplast division took place in the absence of the PDR1 protein. It is therefore likely that PDR1 is involved in the synthesis of the polyglycan filaments. The filaments of the outer PD ring have been observed in multiple species but paralogs of PDR1 have not been identified in genomes of other eukaryotic species that contain plastids. It is not clear whether the paralogs have not been found yet or that the filaments of the outer PD ring are synthesized with other means. [3] [16]

DRP5B/ ARC5

Dynamamin-related protein 5B (DRP5B) is a descendent of the eukaryotic dynamin family and was found in red algae *Cyanidioschyzon merolae*. An ortholog, ARC5, was identified in *Arabidopsis thaliana* by gene cloning analysis. Mutants of ARC5 show faulty chloroplast constriction and resulted in enlarged, dumbbell-shaped chloroplasts. ARC5 and DRP5B resembles the eukaryotic proteins of the dynamin family of GTPases. Eukaryotic dynamin proteins are responsible for endocytosis, involved in cytokinesis and mitochondrial division. Also, prokaryotes do not contain a similar protein, indicating that this dynamin related protein is derived and evolved from the cytokinesis mechanism of the eukaryotic host cell. The function of the DRP5B/ ARC5 protein is a bit similar to the constricting FtsZ ring. DRP5B/ ARC5 is transported from the cytosol to the division site after the FtsZ ring and PD-ring are formed. It is then located at the outside of the envelope membrane and forms a helical structure on the membrane. Instead of the pulling activity of the membrane from the FtsZ ring, the DRP5B constricts the membranes from the outside. DRP5B assembles first as a punctate ring that is laying on top of the outer PD ring. Experiments with *C. merolae* showed that in later constriction, the DRP5B ring repositions between the outer PD ring and the outer envelope membrane and appeared as a connective ring. A study isolated intact constriction complexes, containing the FtsZ ring, PD rings and DRP5B, from the chloroplasts of the red alga *C. merolae* that formed super twisted and spiral structures. When stretched with optimal tweezers up to four times their initial length, they were automatically restored to their original size. In the absence of DRP5B, the complexes did not retract to their original size after stretching. The experiment was also repeated with a depletion of the FtsZ ring and PD rings and results were similar to the complete contractile complexes. This suggest that the dynamin-like contractile ring might be the force for chloroplast constriction. [17] [18] [19]

Regulation of the contractile rings

The FtsZ ring inside the chloroplast and DRP5B ring outside are regulated by two inner envelope membrane proteins (shown in figure 2); ARC6 and PARC6, and two outer envelope membrane proteins; PDV1 and PDV2. [5]

ARC6 is an ortholog of division protein Ftn2 that occurs in cyanobacteria and is therefore derived from the cyanobacterial symbiont. Experiments in *A. thaliana* suggests that ARC6 is involved in the

assembly, stabilization and promoting of the FtsZ ring. Cells lacking ARC6 formed short FtsZ filament particles instead of a ring structure and contained excessively long filaments when this gene was overexpressed. [20] ARC6 is located in the inner envelope membrane at the division site and is involved in tethering the FtsZ ring to the membrane, shown in figure 2a. Analysis in the ARC6 mutant of *A. thaliana* suggests it is not the only agent tethering the FtsZ ring to the membrane. FtsZ1 and FtsZ2 also interacts with ARC3, which determines the positioning of the ring (described in the next paragraph). ARC3 interacts with the inner envelope membrane protein PARC6. FtsZ-PARC6 interaction could provide additional anchoring of the FtsZ ring to the membrane. [21]

PARC6 is a paralog of ARC6. It is suggested that PARC6 is required for the recruitment of PDV1, even though it is not clear if they interact directly. Analysis with PARC6 mutant contain chloroplasts which are irregular in shape and size, include multiple constrictions, excessively long FtsZ filaments and several FtsZ rings. This data indicate that PARC6 prevents FtsZ formation and suggests that it is the reverse version of ARC6, which promotes the formation of the FtsZ ring. [22]

The coordination of the internal and external division components across the two membranes is mediated by an interaction between ARC6 and PDV2, shown in figure 2b. The interaction occurs in the intermembrane space region with the C-terminus of ARC6 and the intermembrane space region of PDV2. [23]

Plastid division protein 1 (PDV1) have been identified in mutant ARC5 analysis of *A. thaliana*. It is an outer envelope membrane protein localized at the midpoint of the chloroplast. Homolog PDV2 was found based on similarity in sequence. PDV1 and PDV2 are outer envelope membrane proteins localized at the midpoint of the chloroplast. DRP5B formation stays in cytosolic patches in cells of PDV1 and PDV2 double mutants. On the other hand, the DRP5B moves to the division site in both single mutants. It showed that PDV1 and PDV2 are both required for the contractile function of DRP5B and interact with each other to promote the recruitment of ARC5/DRP5B to the division site. [24] [25]

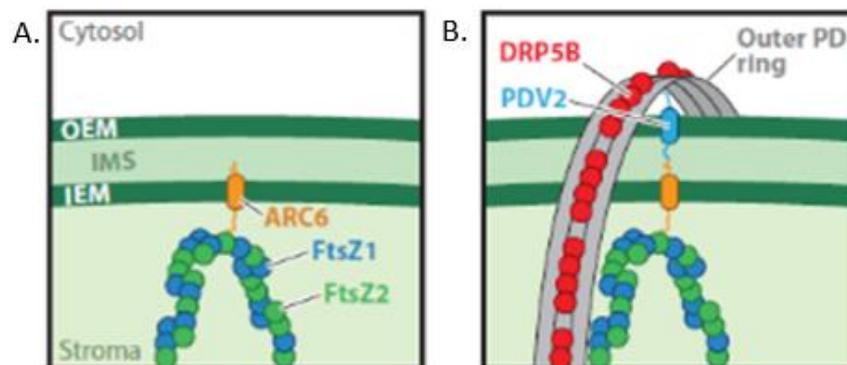


Figure 2: Regulation of the contractile rings by inner envelope membrane (IEM) proteins and outer envelope membrane (OEM) proteins. (A) FtsZ1 and FtsZ2 filaments assemble into the FtsZ ring at the midpoint of the chloroplast and are tethered to the membrane by ARC6. (B) PDV2 is located in the outer envelope membrane and interacts with ARC6 in the intermembrane space (IMS). PDV2 is involved in the recruitment of ARC5/DRP5B from the cytosol. [5]

Positioning of the FtsZ ring directed by the min system

For the cell division to occur at the midpoint of the chloroplast, the components of the division machinery have to know where to be located. This process is called the min system and is derived from the cyanobacterial division mechanism.

The cyanobacterial min system includes minC, minD and minE proteins. MinC is the direct inhibitor of FtsZ filaments formation and it forms a complex with minD. MinD is a peripheral membrane ATPase and can bound ATP. It interacts with the membrane at one part of the cell and forms a pole that attracts minC. Meanwhile, minE interacts with the minD pole and stimulating ATP hydrolysis at minD. ATP hydrolysis disturb the minC-minD complex and releasing it from the membrane. This allows minE to manage the positioning of the minC-minD complex. This results in a concentration gradient of minC that is high near the poles and low at the middle so that the FtsZ ring only forms at the midpoint of the cell. [6]

The mechanism for the FtsZ positioning in chloroplasts is slightly different as it is evolved since the endosymbiotic event. MinD and minE occur in plants and is necessary for chloroplast division. Analysis in *A. thaliana* presented chloroplasts with multiple constriction sites and FtsZ rings in cells lacking minD and overexpressed minE, which resulted in incorrect division-site placement and chloroplasts diverse in shape, size and number. Cells with overexpressed minD and lacking minE showed inhibited FtsZ formation and resulted in few and large chloroplasts with short FtsZ filaments. MinC is not found in chloroplasts, although a minC-like protein have been found in some algae and moss species. Study suggests that ARC3 is involved in the placement of the division site and indicating that is a functional replacement of the minC protein. As an overexpression of ARC3 inhibits the chloroplast division and it resulted in enlarged chloroplasts which is similar with overexpression of bacterial minC. ARC3 also inhibited FtsZ1 and FtsZ2 filaments assembly and was observed as dots and short filaments instead of a ring. These results indicate that the interaction between ARC3 and FtsZ is direct and similar to the bacterial minC protein. [26] [27]

Multiple chloroplast division site 1 (MCD1) protein is also involved in the placement of the FtsZ ring. MCD1 mutants exhibit several FtsZ rings and show similar chloroplast morphologies of the ARC3 mutants. MCD1 overexpression does not inhibit the formation of the FtsZ ring or chloroplast division. It showed that minD was differently localized in the MCD1 mutant. This suggests that MCD1 is involved in transferring minD to the right location. [28]

The red algae *C. merolae* does not encode ARC3 or MCD1 and it is not known how the FtsZ ring is directed at the midpoint of the chloroplast in this species. [4]

Final segregation and formation of the daughter chloroplasts

The precise separation after the constriction of the chloroplast still remains unclear. Two proteins were shown to be involved in the final separation of the daughter cells; CLMP1 and CRL.

Clumped chloroplasts 1 (CLMP1) was identified in an *Arabidopsis thaliana* mutant that caused the chloroplasts to cluster, rather than being dispersed in the cytoplasm. Petioles of CLMP1 mutant observed cells lacking chloroplasts, where normally clustering of the chloroplasts is very common. Chloroplast distribution is actin-dependent in plants, the actin cytoskeleton of the CLMP1 mutant is identical to the wild type. Other organelles showed a normal distribution. Analysis showed that the clustered chloroplasts were connected by thin membranous isthmuses that are characteristic at the late stage of chloroplast division. FtsZ and DRP5B were normally positioned at the midpoint of the clustered chloroplasts. This suggests that the constriction process is unaffected but the separation of

the membranes is impaired. It has been proven that CLMP1 is involved in chloroplast segregation, although the mechanism or specifics have not yet been determined. [29]

The other protein involved in the final chloroplast segregation is Crumpled leaf (CRL). CRL is an outer envelope membrane protein that is conserved in plants and in cyanobacteria, but is not found in other organisms. This indicates a cyanobacterial origin. Cells of the CRL mutant in *A. thaliana* present a reduced number of enlarged plastids, indicating that the chloroplast division was disturbed as a result of segregation defects. Knockout mutants of CRL homologs in the moss *Physcomitrella patens* also showed irregular and enlarged chloroplasts and showed partially similar functions with *A. thaliana* CRL mutant. Time-lapse imaging of chloroplasts in the knockout mutants started to exhibit chloroplast constriction, but failed to segregate and complete the chloroplast division. This suggests that the CRL protein is also needed for the segregation of chloroplast division. [30] [31]

Conclusion

Most of the components of the chloroplast division complex have been identified but some of the mechanisms are still not fully understood. The chloroplast division components that are derived from the cyanobacteria are localized and operating inside the organelle. The origin of the inner PD ring has not been established as the composition is unknown. It is highly likely that the inner PD ring is of host cell origin, because it does not occur in the cyanobacteria. It is interesting that the PD ring is operating within the chloroplast as its origin is probably not of bacterial origin. Although most of the genes derived from bacteria are integrated into the nucleus of the host-cell and need to be transferred from the nucleus to the organelle. This is important because the chloroplast cell division needs to align with the host-cell division cycle.

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