

## IN THIS ISSUE

# Dynamic Trio: FtsZ, Plastid-Dividing, and Dynamin Rings Control Chloroplast Division

Chloroplasts and mitochondria likely evolved from endosymbiosis of bacterial progenitors with a host cell. Thus, it is expected that the division of these organelles bears some resemblance to prokaryotic cell division. However, organelle division is controlled by proteins encoded in the nucleus (host cell genome), and mitochondrial and chloroplast genomes have lost most of the genes necessary for the control of their own division. Some of the nuclear genes involved in organelle division are homologous with bacterial cell division genes, suggesting horizontal gene transfer from endosymbiont to the host genome at some time during their evolutionary history, whereas others are of nuclear origin (Lang et al., 1999).

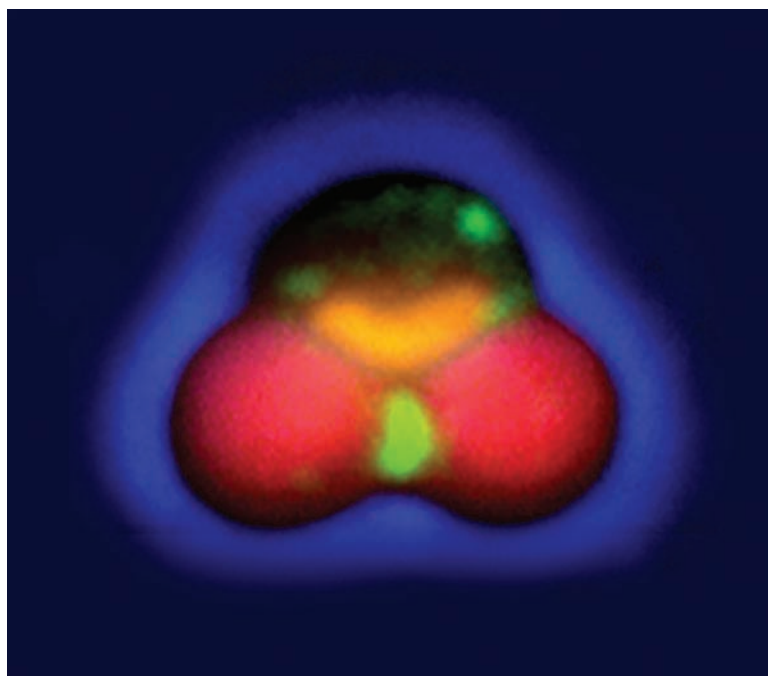
Organelle division is a complex process that is regulated both in concert with and independently of host cell division. During cell division, division of the organelles ensures their inheritance and the maintenance of copy number within progeny cells. Independently of cell division, the copy number of both chloroplasts and mitochondria within an organism varies considerably depending on the cell type, developmental stage, and even environmental conditions. The primitive single-celled red alga *Cyanidioschyzon merolae* is a useful model system for the study of organelle division. Each *Cyanidioschyzon* cell contains a single mitochondrion and a single chloroplast, thus reducing organelle division to a somewhat more simple system than in higher eukaryotes and facilitating experimental control and observation (Figure 1). A complete picture of the organellar dividing apparatuses in this lower eukaryote can be expected to yield important insights into the origin and evolution of these organelles as well as the regulation of their division.

In this issue of *The Plant Cell*, **Miyagishima et al. (pages 655–665)** characterize the

function of a nucleus-encoded dynamin-related protein, CmDnm2, in *Cyanidioschyzon* chloroplast division. This represents an important step forward in our understanding of the mechanism and evolution of chloroplast division. Dynamins, which are not found in prokaryotes, previously were known to be involved in endocytosis and mitochondrial division in eukaryotes and cell plate formation in plants, but little evidence suggested that these proteins were involved in chloroplast division. Rather, it has been assumed that chloroplasts have retained more of a bac-

terial-type division apparatus, a notion supported by the observation that chloroplasts contain numerous homologs of bacterial division genes (Erickson, 2000; Osteryoung, 2001).

For example, *Arabidopsis* contains a family of dynamin-like proteins (ADLs) that are known to function in various membrane scission events. ADL1A is associated with cell plate formation (Kang et al., 2001), ALD6 plays a role in vesicle formation in the *trans*-Golgi network (Jin et al., 2001), and ADL2b appears to be involved in mitochondrial division (Arimura and



**Figure 1.** CmDnm2 Forms a Ring at the Chloroplast Division Site.

Immunofluorescence image of the single-celled red alga *Cyanidioschyzon* showing the CmDnm2 dynamin protein (in green) forming a ring at the chloroplast division site during the late stages of division. The main body of the cell with the nucleus is at top, the single mitochondrion appears in the center of the cell (in orange), and the dividing chloroplast is at bottom. Chlorophyll autofluorescence is shown in red. (Figure courtesy of S. Miyagishima.)

## IN THIS ISSUE

Tsutsumi, 2002). ADL1 has been observed in thylakoid membranes and is hypothesized to play a role in their biogenesis (Park et al., 1998), and ADL2 is localized to plastids and shows high expression in flower tissues (Kang et al., 1998), but dynamins have not been observed previously in association with the chloroplast division apparatus.

### DYNAMINS: EUKARYOTIC MEMBRANE SCISSORS

Dynamins constitute a superfamily of GTPases. However, instead of (or possibly in addition to) functioning as classic “molecular switch” GTPases, dynamins are capable of self-assembling into multimeric ring structures that function in the regulation of membrane scission events, such as those that occur during endocytosis and organelle division. The prototype for dynamin and dynamin-related proteins is mammalian Dynamin1, which self-assembles into stacks of interconnected rings in budding clathrin-coated vesicles that subsequently close and pinch off into newly formed vesicles (Hinshaw, 2000). Most dynamin-related proteins that have been examined readily self-assemble, suggesting a general mechanism of action for this protein family. The precise roles of the GTPase and self-assembly activities of dynamins are unclear, but it appears that self-assembly promotes ring formation and constriction at the division site and stimulates a high rate of GTPase activity, which is required for ring disassembly and subsequent membrane fission (Sever et al., 2000).

### DYNAMIN IN CYANIDIOSCHYZON CHLOROPLAST DIVISION

Chloroplast division is associated with the formation of a so-called FtsZ ring and a series of distinct plastid-dividing (PD) rings (Miyagishima et al., 2001b; Kuroiwa et al., 2002). Two early events in chloroplast division are the accumulation of the GTPase FtsZ and the formation of an FtsZ ring at

the future division site. FtsZ was identified as a cell division protein in *Escherichia coli*, and it plays a central role in regulating the division of most prokaryotic cells (Bramhill, 1997). FtsZ self-assembles into ring structures, like dynamin, but it does not show significant sequence similarity to dynamin. Rather, it has similarity to and has been proposed as a progenitor of tubulin. In plants, FtsZ is a nucleus-encoded chloroplast protein (Osteryoung and Vierling, 1995). The chloroplast FtsZ ring forms in the stroma and faces a second series of electron-dense rings, the PD rings. The PD rings consist of a main outer ring on the cytosolic face of the outer envelope, which is made up of a bundle of unidentified 5-nm filaments (Miyagishima et al., 2001a), an inner ring that faces the FtsZ ring on the stromal side, and sometimes a middle ring between the inner and outer envelope membranes.

Miyagishima et al. show that chloroplast division in Cyanidioschyzon is associated with the formation of a dynamin ring, which forms a third type of ring separate from and in addition to the FtsZ and PD rings. Time-course experiments of the seven phases of chloroplast division, using electron microscopy and immunofluorescence localization of FtsZ and CmDnm2, showed that the FtsZ ring forms before the onset and disassembles during the final stage of chloroplast constriction (phases 2 to 5) and that the PD ring forms and disappears at a somewhat later stage (phases 3 to 6). By contrast, CmDnm2 begins to accumulate in phase 3, after the FtsZ and PD rings have formed, initiates the formation of a ring structure in phase 4, and appears as a constricted ring during phase 5, the final phase of division. The protein often persists in the daughter chloroplasts after division (phase 6) and then disappears before cytokinesis (phase 7).

### ... IN GREEN PLANTS

Miyagishima et al. conducted sequence comparisons of numerous dynamins from

different organisms, which showed that CmDnm2 is related most closely to two previously uncharacterized Arabidopsis proteins, NP\_188606 and BAB02559.1. Intriguingly, Gao et al. (2003) now have shown that NP\_188606 and NP\_188607 (corresponding to loci At3g19730 and At3g19720, respectively) constitute a single dynamin-like protein that is encoded by the gene *ARC5* and that this dynamin plays an important role in chloroplast division in Arabidopsis. Mutants of *ARC5* are defective in chloroplast division and exhibit large dumbbell-shaped chloroplasts. Localization experiments suggested that *ARC5* localizes to a ring at the chloroplast division site positioned on the outer surface of dividing chloroplasts (Gao et al., 2003), which is consistent with the position of the dynamin ring in Cyanidioschyzon chloroplasts. Thus, it appears that this dynamic trio of rings—FtsZ, PD, and dynamin—functions in chloroplast division in higher plants as well as in Cyanidioschyzon.

### ... AND IN MITOCHONDRIAL DIVISION

This discovery has important implications for organelle evolution. It has been hypothesized that dynamin replaced the function of FtsZ in mitochondrial division in higher eukaryotes and that chloroplasts retained a prokaryotic FtsZ-type division, because most higher eukaryotes lack mitochondrial FtsZ and dynamin did not appear to be involved in chloroplast division in higher plants (Erickson, 2000). However, the timing of certain events (e.g., the loss of FtsZ and the acquisition of dynamin) is unclear. Arimura and Tsutsumi (2002) proposed the possibility that the acquisition of dynamin in eukaryotes occurred before the divergence of animals, fungi, green plants, and red algae, whereas the loss of mitochondrial FtsZ occurred separately in green plants and the common ancestor of fungi and animals after the divergence of these groups. In this scenario, therefore, the common ancestor of green plants and red

## IN THIS ISSUE

algae would have used both FtsZ and dynamin in mitochondrial division. Recent work has shown that Cyanidioschyzon (Nishida et al., 2003) and another primitive eukaryote alga, the chromophyte *Malomonas splendens* (Beech et al., 2000), retain the use of FtsZ in mitochondrial division.

The work of Miyagishima et al. together with that of Nishida et al. (2003) shows that Cyanidioschyzon uses FtsZ and dynamin in both mitochondrial and chloroplast division. Therefore, Miyagishima et al. propose that mitochondria and chloroplasts in early eukaryotes likewise exhibited similar division apparatuses that included an FtsZ ring, PD/mitochondria-dividing (MD) rings, and a dynamin ring.

Importantly, in both the mitochondria and chloroplasts of Cyanidioschyzon, the FtsZ ring forms early at the site of future division, before the formation of the PD/MD rings (as it does in higher plant chloroplast division), whereas the dynamin ring appears to form later and to function only in the final separation, after the FtsZ and PD/MD rings have constricted (Miyagishima et al., 2003; Nishida et al., 2003). Thus, the FtsZ, PD/MD, and dynamin rings are shown to have distinct functions in eukaryotic organelle division. This interesting observation is sure to inspire additional experiments aimed at gaining a more complete understanding of the mechanisms of division and the course of organelle evolution in higher eukaryotes.

**Nancy A. Eckardt**  
**News and Reviews Editor**  
**neckardt@aspb.or**

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Nancy A. Eckardt

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