Dynamin: The endosymbiosis ring of power?

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One thing plant cells can't afford to get wrong is chloroplast division. The successful splitting and passing on of chloroplasts to daughter cells is vital to plant cell survival. Indeed, plants (and their progenitors the green algae) have been dividing and passing down their endosymbiotic chloroplasts successfully from generation to generation since the establishment of the chloroplast many hundred of millions of years ago (1). Three new papers now open a new chapter in our understanding of this division (2–4). In this issue of PNAS, Hongbo Gao and colleagues from Katherine Osteryoung's laboratory describe a plant dynamin with a pivotal role in chloroplast division (2). Dyamins are mechanochemical proteins with a range of roles including the pinching off of vesicles (5). Previous work, much of it also from the lab of Osteryoung, had already demonstrated that chloroplasts retain a lot of the division machinery they originally possessed as free-living cyanobacteria. Key bacterial division proteins such as FtsZ, MinE, and MinD also play vital roles in the fission of chloroplasts (6–9). Indeed, bacterial division has served as an excellent model for chloroplast division (10), but this new work forces us to add new components to the model, components derived from the host.

Chloroplasts are no longer free-living, and clearly the host cell controls when fission occurs. A key factor in this control is the fact that the host has confiscated many of the endosymbiont's genes for prokaryotic division proteins. Thus, FtsZ in plant cells is encoded by the nucleus, synthesised on cytoplasmic ribosomes then dispatched into chloroplasts. But, as Gao *et al.* show, the host exerts control over chloroplast division in other ways. In addition to holding many of the genes for the machinery that orchestrates division from within the chloroplast, it has now been shown that the host has added external machinery in the form of a dynamin-like protein known as ARC5 (2).

arc5 (accumulation and replication of chloroplasts) is one of a panel of chloroplast division mutants generated by Kevin Pyke (11). Chloroplasts of *arc5* plants initiate the typical mid-region constriction but often fail to complete the pinching in two, resulting in populations of dumbbell-shaped chloroplasts. Cells of *arc5* plants have between 3 and 15 of these half-divided chloroplasts, whereas a normal wild-type plant cell contains as many as 120 chloroplasts, most of which are spherical to ovoid (11). Gao *et al.* used a clever positional cloning strategy to recover the gene responsible (2). Having tracked the *arc5* locus to a single fragment of *Arabidopsis* genomic DNA cloned into a bacterial artificial chromosome, Gao *et al.* then cut the bacterial artificial chromosome into pieces by using restriction enzymes. Ordinarily, one would try to complement the mutant by introducing these fragments into the mutant genome. The fragment that restored the phenotype would contain the gene being sought. In this case, Gao *et al.* used the isolated DNA fragments to repress the wild-type gene. To do this, they transformed wild-type plants with a library containing antisense fragments derived from the BAC clone with the putative *ARC5* gene in it, and looked for transgenic plants with an *arc5*-like phenotype. In other words they performed a shotgun set of knockouts looking for the one that created the same phenotype as the *arc5* mutant. One fragment, containing a gene (dubbed *ARC5*) with a GTPase domain and the classic signatures of a dynamin (pleckstrin domain and GTPase effector domain), restored the chloroplasts of *arc5* mutant plants to normal. Gao *et al.* then used canonical genetic tools to demonstrate unequivocally that the dynamin-like gene they found is indeed a chloroplast division gene. Thus, a copy of *ARC5* isolated separately from the bacterial artificial chromosome was able to restore the wild-type phenotype, and sequencing revealed that the *arc5* mutant suffered a premature stop codon in the *ARC5* gene, which would produce a drastically truncated protein that must be the cause of incomplete chloroplast division (2).

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By fusing the ARC5 protein gene to jellyfish GFP and expressing the fusion in plants, Gao *et al.* show that ARC5 accumulates in a ring around the mid-region constriction of dividing chloroplast, exactly where it would be expected (9). Interestingly, the GFP-tagged ARC5 protein ring was speckled and did not form a continuous ring encircling the constriction. To find out where ARC5 protein is located exactly, Gao *et al.* then combined isolated chloroplasts with the protein (2). ARC5 attached to the chloroplasts but did not enter, which is consistent with the apparent lack of any targeting peptide, as is carried by nuclear encoded division proteins like FtsZ that enter the chloroplast and work from the inside. Thus ARC5 gathers around the mid region of the chloroplast late in the division phase (9). *arc5* plants fail to complete this fission resulting in accumulation of dumbbell-shaped chloroplasts. This role for dynamins in chloroplast division is highly reminiscent of how another set of dynamins participate in mitochondrial division (12–14).

The recruitment of dynamin in chloroplast division apparently goes well beyond dicotyledenous plants like *Arabidopsis*. Gao *et al.* found a clear homologue of this chloroplast division dynamin in the rice genome (a monocotyledonous plant) (2), and a forthcoming paper (4) describes a homologue from an even more distant "plant," the unicellular red alga *Cyanidioschizon merolae*. Although *C. merolae* lacks many of the elegant genetic tools available to plant biologists with *Arabidopsis*, Tsuneyoshi Kuroiwa has pioneered it as an excellent system to study chloroplast and mitochondrial division. In searches of the red algal genome, Kuroiwa's team found two dynamins, CmDnm1 and CmDnm2, with roles in late fission of the mitochondrion (3) and chloroplast (4), respectively. Interestingly, CmDnm2 shows up in the Japanese group's phylogenetic analysis (4) as the closest relative of Osteryoung's ARC5 protein (also known as AP000417 and the same gene annotated twice as NP 188606), which means that this dynamin subfamily evolved for the role of chloroplast division before the red and green algae diverged, an event at least 0.6 Ga (1 Ga = 1 billion years ago) and perhaps more than 1.2 Ga. This ancient

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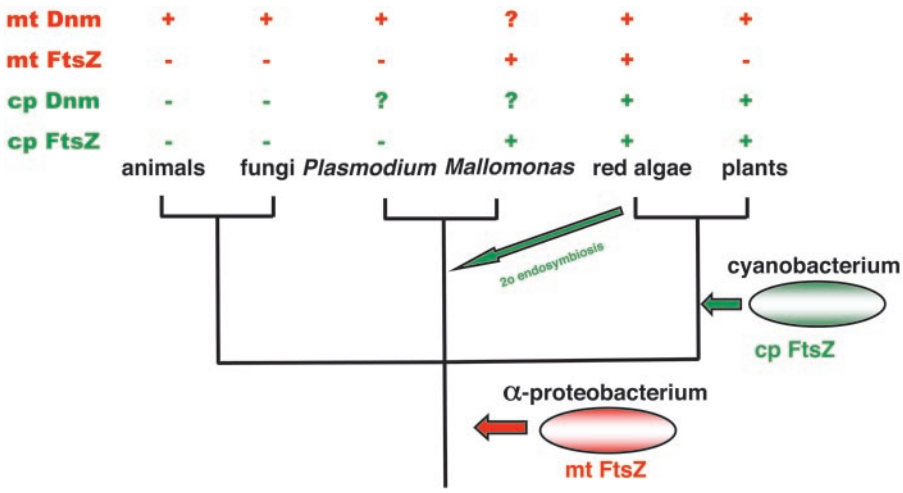

Fig. 1. Known distribution of chloroplast and mitochondrial FtsZ and dynamin proteins in organelle division. Mitochondrial FtsZ was clearly introduced during the endosymbiosis of an alpha proteobacterium to create the mitochondrion. Similarly, chloroplast FtsZ was introduced along with the chloroplast endosymbiosis and then transferred into the lineage containing *Mallomonas* and *Plasmodium* by a secondary endosymbiosis of a red alga. The scheme shows two independent losses of mitochondrial FtsZ (plants and the animal/fungal clade) and another loss of chloroplast FtsZ in the relict plastids of the malaria parasite *Plasmodium*. Chloroplast and mitochondrial dynamins apparently derive from other host dynamins.

enlistment of a eukaryotic protein with a presumed role in generating the mechanical force (but see ref. 15) to pinch off the outer chloroplast membrane at the late stage of fission establishes dynamin recruitment as a key event in the host mastering control of the endosymbiont's division. Intriguingly, the phylogeny now shows that the dynamins recruited for chloroplast division are distinct from those recruited for mitochondrial division (4). Dynamins perform a wide range of membrane pinching roles (constrictase) in eukaryotes (5) and it is not yet clear in dynamin phylogeny exactly what type of dynamin was recruited for the task of finalising organelle division. Nevertheless, given that the acquisition of the mitochondrion preceded the acquisition of chloroplasts it doesn't appear from the trees that the mitochondrial dynamins were coopted for chloroplast division. Otherwise the chloroplast dynamins would appear as a subbranch within the mitochondrial dynamin radiation.

One strength of the *C. merolae* system for organelle division work is that it can be synchronized, and Kuroiwa's team present stunning immunofluorescent localization of the two different dynamin rings assembling around the already formed isthmuses of dividing chloroplasts (4) and mitochondria (3). Furthermore, immunogold electron microscopy demonstrates that these dynamin rings are outside their organelle, on the cytosolic (host) side (3, 4). All of this is consistent with a role for dynamin in completing the organelle division initiated from within by the ancient Fts/Min apparatus inherited from the organelles' bacterial forebears. Importantly, dynamin's action in dividing chloroplasts is distinct both temporally and location wise from the earlier, internal role of FtsZ (4). Thus, dynamin appears to be an adjunct to organelle division rather than a host derived mechanism to replace the FtsZ system (3, 16). Exactly why dynamins are needed for this role is unclear. Hashimoto (17) pointed out that an external cleaving system might have been required when the organelles relinquished their peptidoglycan wall, which in bacteria plays a role in the septum formation. Fortunately, one endosymbiont, the so-called cyanelles of glaucophyte algae like *Cyanophora paradoxa*, still retains a relict peptidoglycan wall around its chloroplast, and the role (if any) for dynamin in conjunction with a walled endosymbiont will be informative.

The current data suggest that dynamin is responsible for latter events in organelle cleavage, whereas FtsZ and the MinDE proteins are responsible for the initiation of cleavage. A puzzling aspect of this situation are the various organelles that apparently lack an FtsZ system. The mitochondria of animals and fungi seem not employ FtsZ as the genes cannot be found in the completed genomes. Similarly plant mitochondria also appear not to initiate their division with FtsZ (18). This suggests that loss of FtsZ mitochondrial division initiation has occurred twice during evolution (Fig. 1). Furthermore, the lack of FtsZ in division of the relict plastids of malaria and *Toxoplasma* parasites (19) also suggests that plastid division can be achieved without this archetypal fission initiation (Fig. 1). Exactly how the organelles determine where the cleavage should occur in these FtsZ and (possibly) MinD/E deficient systems is a mystery. Intuitively, one suspects that an internal mechanism for defining the constriction site (which is typically central) is the most feasible, which perhaps suggests that the host has insinuated a novel system to sense and initiate the division site. Otherwise, one has to

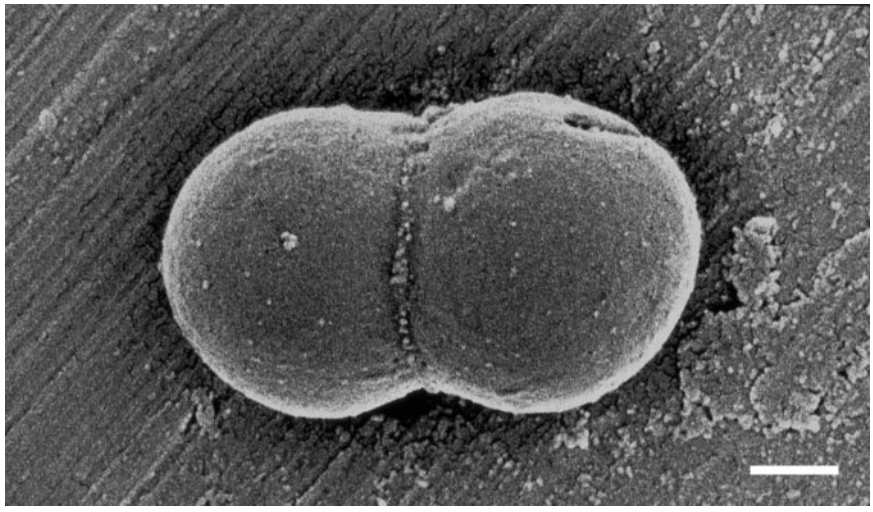

Fig. 2. Scanning electron microscope image of an isolated plastid from *C. merolae* at the late constriction (dumbbell) phase. The outer plastid dividing ring (PD) is clearly visible. New work shows that dynamin assembles around the outside of the plastid at this stage but also suggests that dynamin is not the main constituent of the PD ring. Photo taken by Shin-ya Miyagishima and Tsuneyoshi Kuroiwa. (Scale bar = 500 nm.)

reckon with a completely externally applied cleavage system with the inherent randomness of segregating the all-important organelle genome copies into daughter organelles.

The discovery that dynamin rings finish off chloroplast division opens an exciting chapter in our understanding of endosymbiotic organelle division, but what of the other rings of power? Somewhat surprisingly, the dynamin rings observed around constricting mitochondria and chloroplasts are not the conspicuous external rings so carefully documented by Kuroiwa's group (Fig. 2). These rings, known as the mitochondrial dividing ring (MD) and plastid dividing ring (PD), respectively, were at one time suspected to be composed of dynamin, but the exquisite localization experiments refute that (3, 4). Similarly, it is emerging that the FtsZ ring (where present) is distinct from the inner PD and MD. Thus, there are apparently as many as four rings (FtsZ, inner MD, outer MD, and dynamin) in mitochondria of *C. merolae,* and no fewer than five rings (FtsZ, inner PD, middle PD, outer PD, and dynamin) around the chloroplasts of *C. merolae* (3, 4). Certain of these non-dynamin rings also occur around mitochondria and chloroplasts of other organisms, so their roles must be fundamental in organelle fission (17). We can look forward to learning the composition of some of these rings, soon we expect. In *C. merolae* the outer PD has already been purified and shown to comprise rows of 5-nm filaments containing predominantly a 56-kDa protein (20), so a gene should be found soon. It is also possible that some of these rings are encoded by other *arc* genes, so a genetic approach in *Arabidopsis* might yet contribute to finding more of the lost rings.

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