The Division of Endosymbiotic Organelles

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Mitochondria and chloroplasts are essential eukaryotic organelles of endosymbiotic origin. Dynamic cellular machineries divide these organelles. The mechanisms by which mitochondria and chloroplasts divide were thought to be fundamentally different because chloroplasts use proteins derived from the ancestral prokaryotic cell division machinery, whereas mitochondria have largely evolved a division apparatus that lacks bacterial cell division components. Recent findings indicate, however, that both types of organelles universally require dynamin-related guanosine triphosphatases to divide. This mechanistic link provides fundamental insights into the molecular events driving the division, and possibly the evolution, of organelles in eukaryotes.

Chloroplasts and mitochondria power eukaryotic cells (*1*). Chloroplasts fix carbon from CO_2 into organic molecules that constitute the base of the global food chain. Mitochondria convert the energy stored in these compounds into adenosine triphosphate, the form of cellular energy used to power most of the processes required for growth and development. These organelles (*2*) also have many other metabolic functions essential to eukaryotic organisms (*3*). Mitochondria also play a key role in programmed cell death, a process essential to the development of multicellular organisms (*4*).

Mitochondria and chloroplasts are the descendants of serial endosymbiotic events (*5*). Mitochondria arose first from an α-proteobacterial ancestor that was acquired by either an archaeal or primitive eukaryotic host, and the transition from autonomous bacterium to host (nuclear)-controlled organelle was pivotal in the evolution of eukaryotic cells (*5, 6*). Chloroplasts later arose from a cyanobacterial ancestor acquired by a eukaryote in which mitochondria were already established (*7*). Most of the bacterial genes were transferred to the nuclear genome or lost as the endosymbionts were subjugated by the host cell (*8*), but both organelles in present-day eukaryotes retain genes, metabolic activities, genetic mechanisms, and protein import complexes that clearly reflect their prokaryotic origins.

Like their free-living ancestors, both chloroplasts and mitochondria divide. Organelle division, segregation, and growth are often uncoupled from the cell division cycle, indicating that organelle and cell division are independent processes (*9, 10*). Division of mitochondria and chloroplasts is orchestrated by multicomponent protein machines that assemble and drive the constriction and fission of the organellar membranes. Because both organelles are surrounded by inner and outer membranes that differ in composition, their division machines must accomplish the synchronized constriction of both membranes, the subsequent fusion of the four lipid bilayers, the final separation of the two daughter organelles, and possibly the resolution of the fused membranes back into two discrete bilayers (*11, 12*) (Fig. 1).

Chloroplasts in the most highly evolved photosynthetic eukaryotes still divide using components derived from those used for cell division in their prokaryotic ancestors. In contrast, mitochondria in fungi, plants, and animals appear to have lost division components of bacterial origin, although some primitive eukaryotes have retained mitochondrial FtsZ (described below) (*13–15*). At present, with some of the key players identified, we are beginning to elucidate the biochemical mechanisms governing organelle division. Insight into these mechanisms has been aided in part by our recent understanding of both the similarities and differences in how chloroplasts and mitochondria divide in eukaryotes.

Pivotal Roles of FtsZ and Dynamin-Related Proteins in Organelle Division

Two types of self-assembling guanosine triphosphatase (GTPase) proteins are critical for organelle division (Table 1). The first is related to the bacterial cell division protein FtsZ. FtsZ is a prokaryotic cytoskeletal protein localized in the cytoplasm, and is structurally and evolutionarily related to the eukaryotic tubulins (*16*). Before cell division in bacteria, FtsZ—the earliest acting component of the division machinery—assembles into a membrane-tethered ring at the mid-cell division site. The FtsZ ring, which constricts as division progresses, probably serves as a scaffold for the recruitment of additional cell division proteins to the cell center, and may also function in a GTPase-dependent manner to generate the force required for membrane deformation and constriction (*16*).

A role for FtsZ in organelle division became evident when a nuclear gene encoding a chloroplast-targeted form of the protein was identified in the model plant *Arabidopsis thaliana* (*17*). The plant *FtsZ* gene evolved from a related gene present in the cyanobacterial predecessor of chloroplasts. It is now well established that FtsZ is a key structural component of the chloroplast division machinery in perhaps all photosynthetic eukaryotes (see below). Recently, FtsZ proteins related to those in α-proteobacteria have also been identified in several primitive eukaryotes, and they function in mitochondrial division in one such organism (*13–15*). The chloroplast (Figs. 1 and 2) and mitochondrial forms of FtsZ assemble into inner membrane–associated rings in the stromal and matrix compartments, respectively (*18*), which are topologically equivalent to the prokaryotic cytoplasm.

Given the prokaryotic origin of chloroplasts and mitochondria, it was perhaps not surprising to discover that host cells recruit FtsZs to function as organelle division proteins. What was surprising was the absence of α-proteobacteria–related *FtsZ* genes in the sequenced genomes of model fungi, animals, and plants, indicating that FtsZ no longer played a role in mitochondrial division in these lineages. The first hints that components other than FtsZ-like proteins were required for mitochondrial division came from studies in *Saccharomyces cerevisiae* and *Caenorhabditis elegans* (*19, 20*), which led to the discovery that a second group of large self-assembling GTPases, the dynamin-related proteins (DRPs), function in mitochondrial fission in these organisms (*10, 21–23*) (Fig. 3).

In contrast to FtsZs, which appear to function only in cell and organelle division, DRPs participate in diverse cellular processes (*24*). The prototypic member of this family, dynamin, mediates the scission of clathrin-coated pits from the plasma membrane during endocytosis [reviewed in (*25*)]. In vitro, dynamin self-assembles into

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spiral-like filaments that bind to liposomes and remodel them into tubules, which constrict and divide upon addition of GTP (*26, 27*). GTP hydrolysis also effects an increase in the spacing between dynamin rings within a filament (*28*). On the basis of these activities, dynamin has been postulated to play a mechanochemical role in severing endocytic vesicles from the plasma membrane. Although there is compelling evidence that dynamin acts directly as a mechanochemical transducer (*29*), it may also function as a classical signaling GTPase that recruits downstream effectors responsible for membrane severing (*30–32*).

Differences Between FtsZ-Dependent and -Independent Organelle Division

The finding that DRPs, but not FtsZs, mediate mitochondrial fission in fungi, plants, and animals initially suggested that a distinct DRP-dependent mitochondrial division mechanism evolved in higher eukaryotes to replace the FtsZ-based system present in primitive eukaryotes. However, DRPs also function in the division of FtsZ-bearing mitochondria and chloroplasts, and organelle division–associated DRPs exist in all major eukaryotic groups (*10, 21–23, 33, 34*). Thus, FtsZs function only in the division of some organelles, whereas DRPs seem to be required universally for organelle division. This raises the intriguing possibility that DRPs were acquired during, and are signatures of, the endosymbiotic events that triggered organelle evolution.

The roles played by FtsZs and DRPs in organelle division are distinct (Fig. 1). In organelles requiring both FtsZ and a DRP, assembly of an FtsZ ring near the inner membrane surface initiates the division process (*34, 35*). The FtsZ ring probably marks and recruits other proteins to the division site. In contrast, DRPs assemble at the division site only after most of organelle constriction has been accomplished, and they remain there until the membranes are severed (*36–38*). Thus, FtsZs act early and DRPs act late during organelle division. Moreover, FtsZs and DRPs associate with and function on different membranes; FtsZs associate with inner organellar membranes, whereas DRPs associate with outer organellar membranes (*13, 15, 19, 20, 23, 33, 35–37, 39, 40*) (Fig. 1).

The late function of DRPs in FtsZ-dependent organelle division parallels that of dynamin, which during endocytosis acts after most of the membrane constriction is accomplished by the assembly of clathrin onto the cytosolic surface of the plasma membrane (*41*). Given the small diameter of the spiral-like structures that DRPs form as a general property, it may be that DRPs can function in the fission of membranes only after some constriction has occurred. The loss of the α-proteobacteria–like FtsZ proteins from higher eukaryotes may reflect the fact that mitochondria have become more dynamic, reticular, and heterogeneous during evolution and have perhaps acquired independent mechanisms for initial mitochondrial constriction.

Alternatively, mitochondrial FtsZs may have been replaced in higher eukaryotes by one or more functionally equivalent components inside the organelle. However, all the mitochondrial division proteins identified to date are outer membrane–associated, which suggests that mitochondrial division does not require inner membrane–associated components (*12*). In yeast mutants lacking DRP-dependent mitochondrial fission, the force generated during cytokinesis is sufficient to divide mitochondrial tubules that extend from mother to daughter cell in a manner that preserves mitochondrial compartment integrity (*42*). Mitochondrial inner membrane fission occurs independently of outer membrane fission in *C. elegans* (*21*); hence, if machinery exists to regulate the dynamics of the inner membrane, it may have evolved independently to function in the formation and regulation of inner membrane cristae structures rather than in mitochondrial division per se.

Origin of DRPs Involved in Organelle Division

There is little question that the FtsZ proteins required for mitochondrial and chloroplast division evolved from those in the cell division machineries of their respective α-proteobacterial and cyanobacterial ancestors. The origins of the dynamin-related organelle division proteins are less clear. All DRPs contain three hallmark domains: the GTPase, middle, and assembly or GTPase effector (GED) domains (*24*). Phylogenetic analysis among the members of this family indicates that distinct groups have evolved in eukaryotic cells to function in such diverse processes as endocytosis, membrane trafficking, chloroplast division, mitochondrial division, mitochondrial fusion, plant cell plate formation, and resistance to viral infection (*24*, *34*).

The recent sequencing of the genome from the ancient protozoan *Giardia intestinalis* (*43*) may shed some light on the origin of DRPs dedicated to organelle division. *Giardia* is thought to lack mitochondria, but the

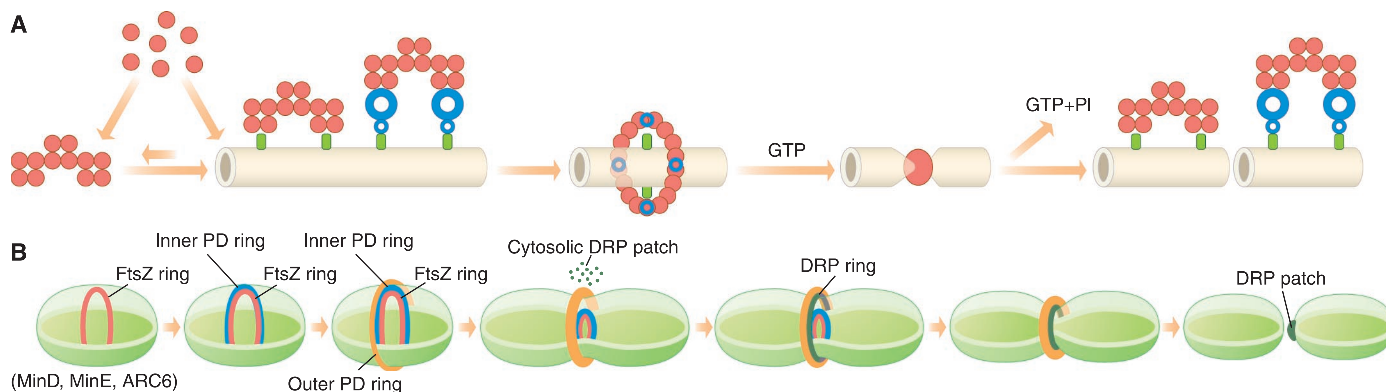

Fig. 1. Molecular pathways for chloroplast and mitochondrial division. (**A**) Model for mitochondrial division in yeast (*12, 73–77*). The behavior of the DRP Dnm1 (red) relative to a mitochondrion during fission is depicted as a linear pathway. The relative rate for each reaction is indicated by different arrow lengths. The proposed interactions among Dnm1, Fis1 (blue), and Mdv1 (green) are indicated during each step of the pathway. Dnm1 self-assembles into punctate structures in a mitochondria-dependent manner. These structures associate with the mitochondrial outer membrane in a dynamic manner. Mdv1 also is associated with the outer membrane, but in a stable manner, via an interaction of its N-terminal domain with the cytosolic TPR-like domain of Fis1. After Dnm1 structures are targeted to the membrane, Mdv1 coassembles into these structures via its C-terminal WD domain and may prevent Dnm1 GTP hydrolysis to allow Dnm1 structures to remodel into an active fission apparatus. In a manner that is dependent on an interaction between Mdv1 and Fis1, fission is triggered. (**B**) Model for chloroplast division in plants, based on recent studies (*11, 33–36, 39, 53, 54, 62, 66–68*). The FtsZ ring assembles first, mediated by the activity of MinD, MinE, and ARC6. Sequential assembly of the inner and outer PD rings follows. Constriction commences, during which the FtsZ and inner PD rings maintain their thickness by loss of components while the outer PD ring gains thickness. The plastid division DRP is recruited from cytosolic patches to the division site at a late stage of constriction. The FtsZ, inner PD ring, and outer PD ring disassemble (in sequence) and the daughter plastids separate. The DRP persists for a time on one of the newborn organelles after their separation is complete.

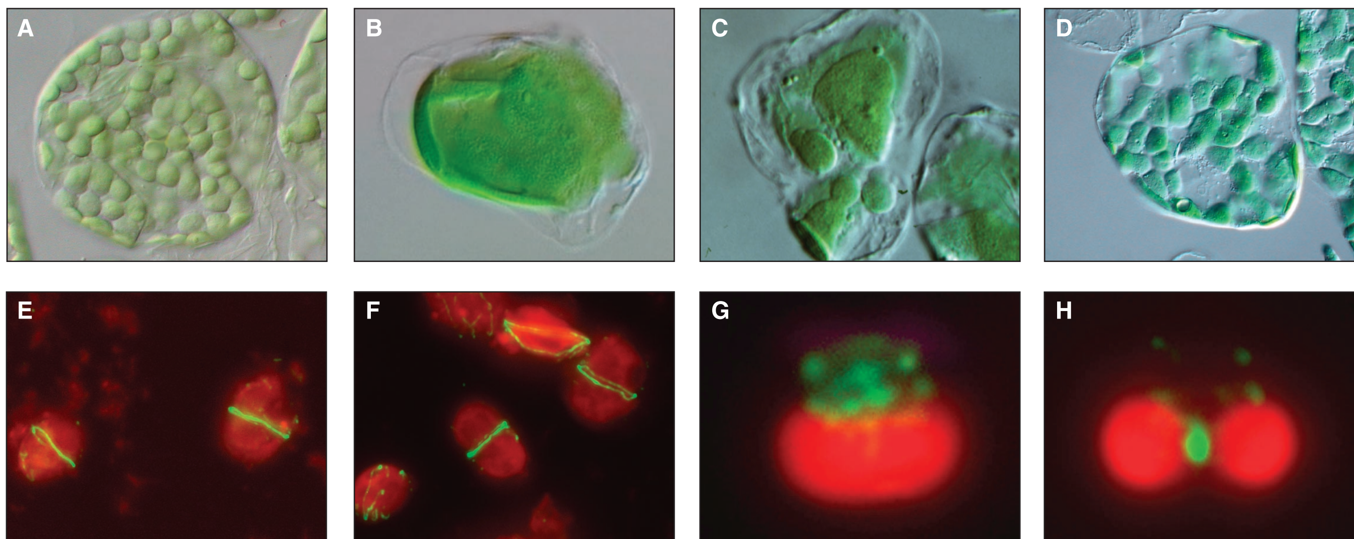

Fig. 2. Chloroplast morphology and division component organization and dynamics. (**A** to **D**) Chloroplasts in *A. thaliana* leaf cells from wild-type plants (A) and plants expressing antisense transgenes for FtsZ2 (B), MinD (C), or the DRP ARC5 (D). The chloroplasts in FtsZ1 antisense and ARC6 mutant plants look similar to those in FtsZ2 antisense plants. Magnifications, ×350 to ×370. (**E** to **H**) Visualization of chloroplast division components. (E and F) Immunofluorescence detection of FtsZ1 (E) and FtsZ2 (F) rings in *A. thaliana*. Images were processed as described (*53*). ARC6-GFP and ARC5-GFP (GFP, green fluorescent protein) are also detected at the division site. (G and H) Immunofluorescence detection of the DRP CmDnm2 in the unicellular red alga *C. merolae* (*36*). CmDnm2 localizes to cytosolic patches after constriction of the single *C. merolae* chloroplast commences (G). Subsequently, it is recruited to the division site (H). In all panels, red chlorophyll autofluorescence reveals the shape of the chloroplast. Magnifications, ×1000 to ×7000.

amitochondriate feature of *Giardia* is the result of secondary mitochondrial loss (*6*). The *Giardia* genome contains only a single gene encoding a DRP localized to internal vesicular structures, in contrast to other ancient eukaryotes that also have mitochondrial DRPs (*37, 44*). Comparison of the *Giardia* DRP to those in other organisms indicates that it is most similar to the fungal Vps1 group, which in budding yeast functions in the biogenesis of Golgi-derived vesicles and the division of peroxisomes (*45, 46*). The Vps1 family is most similar to the DRPs that function in mitochondrial division (*24, 34*). These observations suggest that the eukaryote progenitor had a single DRP that, through gene duplication and divergence, produced DRP families that function in organelle division and other cellular processes. They also point to the central role that DRPs may have played in the evolution of the eukaryotic cell.

Large GTPases distantly related to the eukaryotic DRPs are encoded in some bacterial genomes (*24, 47*), although their functions have not been investigated. As shown for the eukaryotic tubulin and actin families, which evolved from the bacterial FtsZ and MreB families, respectively (*48*), we may learn in time that DRPs also have structurally and evolutionarily related counterparts in prokaryotes from which they ultimately derived.

Mitochondrial and Plastid Dividing Rings

In addition to the FtsZ and DRP rings, mitochondria in primitive eukaryotes and chloroplasts in most photosynthetic eukaryotes have electron-dense structures termed mitochondrial dividing (MD) rings and plastid-dividing (PD) rings, respectively, that are detectable by electron microscopy (EM) during organelle division [reviewed in (*34*)] (Fig. 1B). EM images indicate that these rings are composed of two concentric ring structures, one positioned on the stromal or matrix surface of the inner organellar membrane and the other on the cytosolic surface of the outer membrane. The compositions of the MD and PD rings are not known, although EM studies suggest that they are distinct from the FtsZ and DRP rings and that they assemble after the FtsZ ring but before the DRPs are recruited to the division site (*33–37*). MD rings are not detectable in dividing mitochondria of higher eukaryotes, whereas PD rings are widespread in photosynthetic eukaryotes. Current evidence suggests that they may be restricted to organelles in which FtsZ plays a role in division. Because related structures have not been detected in bacteria, it has been suggested that the organelle dividing rings were inventions of the endosymbiotic host cell and that, like FtsZ, the MD ring was lost from fungal, plant, and animal mitochondria (*34*). The functions of the MD and PD rings in organelle division are currently unknown.

The Chloroplast Division Machinery

In addition to the eukaryotically derived plastid division DRP described above, six plastid division proteins of prokaryotic origin have been identified to date in plants (Table 1). These include FtsZ1 and FtsZ2, which are structural components of the division machinery; ARC6 and ARTEMIS, which appear to function in assembly of the division complex and are found only in plants and cyanobacteria; and MinD and MinE, which mediate positioning of the chloroplast division site (Fig. 1B). These six proteins are encoded in the nucleus and targeted to the chloroplast by N-terminal transit peptides.

Plants and green algae have two phylogenetically distinct forms of FtsZ, FtsZ1 and FtsZ2, both of which function in plastid division [(*49–52*) and references therein]. The two proteins colocalize to mid-plastid rings (Fig. 2, E and F) that reside together in the stromal compartment (*17, 39, 40, 53*). Recent data suggest that they coassemble in a complex (*54, 55*). Both are predicted to be polymer-forming GTPases (*51*), and FtsZ1 has been shown to undergo GTP-dependent multimerization in vitro (*56*). FtsZ1 and FtsZ2 probably have nonredundant functions (*49, 51, 52*).

The reason for the evolution of two forms of FtsZ in plants when most bacteria have only a single FtsZ remains unclear. Numerous conserved differences between the protein families have been identified (*51, 52, 57*), the most notable of which is the presence in FtsZ2 and absence in FtsZ1 of a short, conserved region at the extreme C terminus called the C-terminal core domain (*58*). This region is also found in most bacterial FtsZ proteins, and in *Escherichia coli* it is required for the interaction of FtsZ with two other cell division proteins, ZipA and FtsA [reviewed in (*59*)]. Neither ZipA nor FtsA can be identified in plants (or cyanobacteria) on the basis of sequence similarity, but the conservation of the C-terminal core domain in FtsZ2 proteins suggests the presence of functionally related

chloroplast division proteins in plants and green algae that interact specifically with FtsZ2. The presence or absence of the C-terminal core domain may thus define an important functional difference between FtsZ1 and FtsZ2. Because the plastid division activity of these proteins is dose-dependent (*53, 60, 61*), rigorous testing of this hypothesis by genetic manipulation will also require careful manipulation of total FtsZ protein levels.

The recent identification of a new plastid division gene, *ARC6* (*54, 62*), suggests that molecular chaperones may function in chloroplast division. Chaperones participate in many cellular processes by regulating the conformational states of the participant proteins (*63*). ARC6 and the related cyanobacterial cell division protein Ftn2 bear a conserved region resembling the J domains of DnaJ-related proteins (*54, 64*), which interact with specific Hsp70 chaperone partners and stimulate their adenosine triphosphatase activity (*63*). ARC6 spans the inner chloroplast membrane at the division site, with its J domain–like region exposed to the chloroplast stroma where FtsZ1 and FtsZ2 reside. Changes in *ARC6* expression levels perturb FtsZ filament morphology (*54*). ARC6 could thus be part of a chaperone system of cyanobacterial origin that promotes the formation of FtsZ polymers during chloroplast division.

In addition to the inner and outer membranes, chloroplasts contain an internal system of membranes, the thylakoids, which are partitioned between the two daughter plastids during division (*9*). Normally, chloroplast and thylakoid division are coupled. However, mutations in the gene encoding the protein ARTEMIS disrupt inner and outer membrane fission but not thylakoid constriction and partitioning (*65*). This indicates that the two processes are distinct and suggests that ARTEMIS plays a role in their coordination. ARTEMIS is localized in the inner membrane and has similarity to the YidC/Oxa1p/Alb3 family of membrane integrases. Because the latter proteins participate in assembly of membrane protein complexes, it has been hypothesized that ARTEMIS functions in assembly of the chloroplast division apparatus (*65*).

Chloroplasts usually divide in the middle, implying that assembly of the division apparatus is spatially regulated. Placement of the chloroplast division site in plants is mediated in part by MinD and MinE (*66–68*). The related proteins in bacteria are components of a dynamic system that controls the site of FtsZ ring formation and hence of cell division. In *E. coli*, MinD functions by oscillating between the two cell poles, carrying along as cargo MinC, an inhibitor of FtsZ ring formation [reviewed in (*59*)]. MinE also oscillates, sweeping the MinC-MinD complex away from the cell center. Collectively, these activities allow the FtsZ ring to assemble only at the cell center. In plants, changes in chloroplast MinD (Fig. 2C) or MinE levels produce abnormalities in constriction sites, FtsZ localization patterns, and chloroplast size and shape consistent with a role in regulating the site of FtsZ ring assembly during chloroplast division (*54, 66–68*). Both proteins localize in a manner consistent with oscillatory behavior, similar to their bacterial counterparts (*66*). By establishing the site of FtsZ ring assembly, MinD and MinE may also affect positioning of the PD and DRP-containing rings. MinC, although present in cyanobacteria as well as other prokaryotes, has not been identified in plants, which suggests that a divergent but functionally similar protein has replaced it or that MinC-like activity is not required in plants.

The plastid-associated DRP, called ARC5 in plants and CmDnm2 in the primitive red alga *Cyanidioschyzon merolae*, is the only plastid division protein identified thus far that is eukaryotic in origin and functions from outside the organelle (*33, 36*). Like other DRPs, these proteins act late in the fission process. In *C. merolae*, a cytosolic pool of CmDnm2 is recruited to the plastid division site after sequential assembly of the FtsZ ring, inner PD ring, and outer PD ring, and after the initiation of constriction (*36*) (Fig. 2, G and H). This behavior is similar to that of the yeast mitochondrial DRP Dnm1 (described below). Discovery of these chloroplast DRPs revealed that the plastid division machinery is of mixed evolutionary origin and established a mechanistic link between chloroplast and mitochondrial division.

The Mitochondrial Division Machinery

Molecular mechanisms for mitochondrial fission in yeast and animals have begun to emerge and are remarkably similar, but some interesting differences are also apparent (*12, 69*). In all cell types examined, the mitochondrial fission DRP, called Dnm1 in yeast and Drp1 (or Dlp1) in animals, assembles to form punctate structures that are both extramitochondrial and associated with the cytosolic face of the mitochondrial outer membrane (*10, 20–22, 38, 70, 71*) (Fig. 3, E and F). The majority of Dnm1 is present in these structures, whereas the majority of Drp1 in animal cells appears to be unassembled in the cytosol, possibly reflecting differences in the components that regulate the self-assembly of these DRPs (*10, 20*) (Fig. 3, E and F). On the outer membrane, a subset of Dnm1- or Drp1-containing puncta are directly associated with sites of mitochondrial constriction and fission (*10, 21, 22, 72*), suggesting a multistep pathway for mitochondrial fission in which Dnm1 or Drp1 structures assemble, are targeted to mitochondria, and, at a rate-limiting step in the pathway, mediate the division of the mitochondrial membranes (Fig. 1A).

At least two proteins in addition to Dnm1 (Table 1) function in the mitochondrial fission pathway in yeast (*73–76*) (Figs. 1A and 3). One is the small integral outer membrane

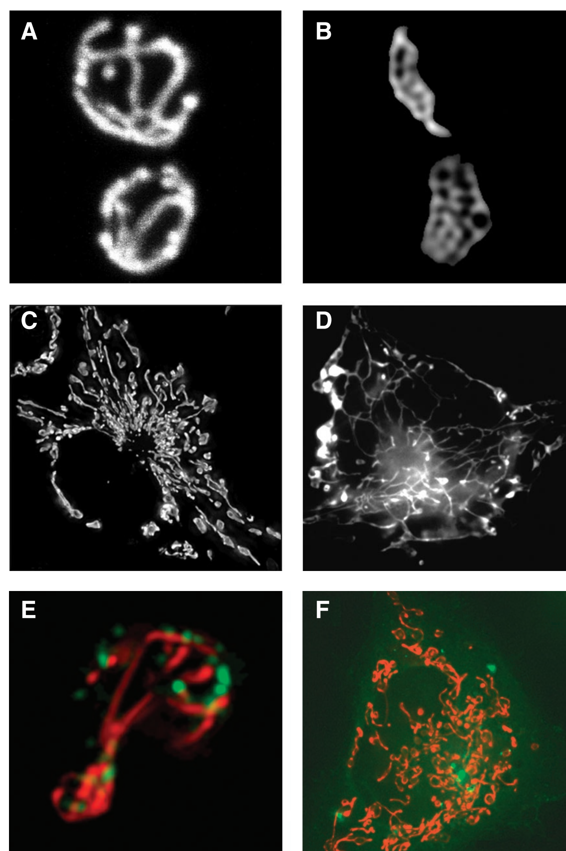

Fig. 3. Mitochondrial morphology and division component organization and dynamics. (**A** to **D**) Mitochondria in *S. cerevisiae* (A) and in mammalian COS-7 cells (C) are reticular structures. Disruption of mitochondrial fission in yeast (B) and COS-7 cells (D) leads to the formation of highly interconnected mitochondrial tubular structures, resulting from unopposed mitochondrial fusion, which indicates that a balance between fission and fusion events is required to maintain normal mitochondrial morphology. (**E** and **F**) Visualization of mitochondrial division components. Mitochondrial division DRPs are present in punctate structures associated with mitochondria in *S. cerevisiae* [(E); Dnm1-GFP in green, mitochondria in red] and in mammalian COS-7 cells [(F); Drp1-GFP in green, mitochondria in red]. In mammalian cells, there is also a substantial pool of unassembled cytosolic Drp1. The yeast mitochondrial division component Mdv1 colocalizes with Dnm1 punctate structures on the mitochondrial membrane. In contrast, Fis1 is uniformly distributed on the mitochondrial outer membrane in yeast and animal cells. Magnifications, ×500 to ×1500.

Table 1. Identified components of the organelle division machineries.

Protein	Process	Evolutionary origin	Reported distribution in eukaryotes	Name, activity of related proteins	Suborganellar localization/topology	Comments	References
FtsZ1, FtsZ2	Chloroplast division	Cyanobacterial endosymbiont	Plants, green algae	FtsZ; forms a ring at the bacterial cell division site	Medial ring, stromal side of inner membrane		(*49*, *50*, *53*)
FtsZ-cp	Chloroplast division	Cyanobacterial endosymbiont	Red and chromophyte algae	(same as above)	Medial ring, stromal side of inner membrane	Phylogenetically distinct from FtsZ1 and FtsZ2	(*13*, *14*, *35*)
FtsZ-mt	Mitochondrial division	α-Proteobacterial endosymbiont	Primitive eukaryotes	(same as above)	Medial ring in matrix		(*13–15*, *35*)
ARC5/ CmDnm2	Chloroplast division	Eukaryotic host	Plants, red algae		Cytosolic patches, medial ring on cytosolic side of outer membrane	Member of DRP family	(*33*, *36*)
CmDnm1	Mitochondrial division	Eukaryotic host	Red algae	Dnm1	Cytosolic patches, medial ring on cytosolic side of outer membrane		(*37*)
Dnm1, Drp1 (Dlp1) ADL2b	Mitochondrial division	Eukaryotic host	Fungi, animals, plants		Assembled structures interact with sites on cytosolic side of outer membrane associated with constriction; cycles on and off the membrane	Dnm1/Drp1 interacts with itself; Dnm1 interacts with Mdv1; Drp1 interacts with hFis1 and Erp-1	(*10*, *19*, *20–22*, *76–79*, *83*)
Mdv1	Mitochondrial division		Budding yeasts		Peripheral outer membrane protein; cytosolic surface, colocalizes to Dnm1 assembled structures	Interacts with Dnm1 and Fis1	(*74*, *77*, *78*)
Erp1	Mitochondrial division		Fungi, animals	Related to endophilins	Colocalizes to Dnm1 assembled structures		(*83*)
Fis1, hFis1	Mitochondrial division		Fungi, animals		C-tail anchored outer membrane, TPR-like domain exposed to cytosol; uniform outer membrane distribution	Fis1 interacts with Mdv1; hFis1 interacts with Drp1	(*76*, *77*, *79*, *80*, *82*)
ARC6	Chloroplast division	Cyanobacterial endosymbiont	Plants	Ftn2; cell division in cyanobacteria	Medial ring, spans inner membrane, J domain exposed to stroma	May function in assembly or stabilization of the FtsZ ring	(*53*, *62*)
ARTEMIS	Chloroplast division	Cyanobacterial endosymbiont and eukaryotic host	Plants	ARTEMIS-like protein; cell division in cyanobacteria	Spans inner membrane	Choroplast division but not thylakoid partitioning impaired in mutant; similar to YidC/Oxa1/Alb3 family; also has receptor kinase–like region	(*65*)
MinD	Chloroplast division	Cyanobacterial endosymbiont	Plants, green algae	MinD, FtsZ ring positioning in some bacteria	Dynamic localization in stroma		(*66–68*)
MinE	Chloroplast division	Cyanobacterial endosymbiont	Plants, green algae	MinE, FtsZ ring positioning in some bacteria	Dynamic localization in stroma		(*66*, *68*)

protein Fis1, the majority of which faces the cytosol (*76*). The other is Mdv1, a peripheral outer membrane protein with at least three distinct regions required for fission that mediate different protein interactions: an N-terminal extension that interacts with Fis1, a central coiled-coil region that mediates the formation of an Mdv1 dimer, and a C-terminal, seven-bladed WD repeat domain

that interacts with Dnm1 (*73–75, 77, 78*). Genetic, biochemical, and cytological data cited above suggest a model for mitochondrial fission in yeast (Fig. 1A) wherein assembled Dnm1 structures are targeted to the mitochondrial membrane in a manner that is partly dependent on Fis1, but may also depend on the intrinsic ability of Dnm1 to interact independently with mitochondria. Once targeted to the mitochondrial membrane, Mdv1 assembles into Dnm1-containing structures and likely functions to regulate their conformation and assembly by influencing Dnm1's guanine nucleotide binding state. Within these Dnm1-Mdv1 structures, an interaction between Mdv1 and Fis1 transmits a signal required for mitochondrial membrane severing.

Fis1 homologs have also been identified in animals. Recent studies indicate that, as in yeast, Fis1 in animals is required for mitochondrial fission, and that it interacts directly with Drp1 in vitro (*79, 80*). The mouse Fis1 cytosolic domain is superhelical, consisting of three pairs of two antiparallel α helices related to tetratricopeptide repeat (TPR) domains, which are known to facilitate specific protein-protein interactions in functionally diverse processes (*81, 82*). In TPR proteins, pairs of antiparallel α helices fold into a superhelical structure with a groove that constitutes a binding site for specific protein partners. Thus, the structural data suggest that Fis1 functions as a protein interaction platform at two points in mitochondrial division: early as a DRP receptor, and late as a catalyst via its interaction with Mdv1 to sever mitochondrial membranes.

Mdv1 homologs have not been found in organisms other than budding yeasts, which raises the possibility that Mdv1 function has been replaced by another component in other eukaryotes. In *C. elegans*, Erp-1, a homolog of mammalian endophilin B, coassembles with Drp-1 on mitochondria and is required for mitochondrial division, similar to Mdv1 (*83*). A related protein, endophilin-1, functions with dynamin during endocytosis and has been shown in vitro to coassemble with dynamin into rings on lipid tubules and to inhibit dynamin's GTP-dependent vesiculating activity (*84, 85*). Also similar to Mdv1, both endophilin-1 and endophilin B have an adaptor-like domain organization consisting of an N-terminal domain responsible for lipid binding and lysophosphatidic acid–acyl transferase activity, a central coiled-coil domain for oligomerization, and a C-terminal Src homology 3 domain responsible for binding to dynamin (*84, 86*). Erp-1/endophilin B proteins in animals thus appear to function similarly to Mdv1 in yeast, and they may act to directly facilitate membrane constriction during mitochondrial fission.

Perspectives

Although much progress has been made, many questions concerning the mechanisms of organelle fission remain. What is the mechanistic basis of the need for both FtsZ and DRPs in the division of some organelles and not others? At the heart of this question are the precise functions and in vivo structures of the assembled FtsZ ring and DRPs. For example, the diameter of the spiral formed by dynamin is considerably smaller than the diameters of chloroplasts and mitochondria, which are both larger than that of the endocytic vesicles on which dynamin acts. Have different DRPs been tailored in terms of their structural properties to accommodate the constriction of membranes of different diameters? Are FtsZs and DRPs in fact force-generating, and if so, how is this force coupled to membrane constriction? Related to this question is the intriguing finding that chloroplast division seems to require multiple inner membrane–associated proteins, whereas mitochondrial division in fungi, animals, and plants seems to require none. This raises the question of just how similar the processes of chloroplast and mitochondrial division really are. Are there fundamental mechanistic differences, or is mitochondrial division in higher eukaryotes simply a more highly evolved form of organelle division that requires minimal machinery? In this context, it may be important that mitochondria are evolutionarily much older than chloroplasts. Understanding how constriction and fission of the organellar membranes are accomplished will require more detailed analyses of the division components. Such analyses would be aided considerably by systems, not yet developed, that recapitulate organelle division in vitro. Work on understanding how other intracellular organelles divide and on how bacterial cell division occurs will also continue to inform mechanistic studies of chloroplast and mitochondrial division.

A broader issue concerns the physiological functions of the organelle division machineries. Because organelles perform critical metabolic functions and do not arise de novo, both their genomes and at least a fraction of their membranes must be transmitted in some way during mitosis. Thus, organelle propagation seems one obvious function. Consistently, the embryonic lethality resulting from loss of DRP-mediated mitochondrial division in *C. elegans* suggests that an active division machinery is essential for mitochondrial propagation in this organism (*21*). However, mitochondria in yeast and chloroplasts in plants can be propagated to daughter cells in the apparent absence of a functional organelle fission machinery (*19, 49, 62*). Active organelle division may thus not be strictly required in all dividing cells solely for organelle propagation, although it probably enhances the efficiency of the process by increasing organelle numbers and distributing organellar membranes more uniformly in cells. Because its placement can be regulated, an active organelle division apparatus might also promote efficient genome segregation during division of the organelles. Organelle DNA replication and mitochondrial nucleoid location and segregation are unrelated to the sites of organelle division, however (*9, 42, 87, 88*). The uncoupling of nucleoid segregation from mitochondrial division in animal and yeast cells may have resulted from the loss of bacterially derived division components, such as FtsZ and MinC, that spatially regulate the placement of the division site. However, it is not known whether the position of the mitochondrial division apparatus in these organisms is regulated with respect to some as yet unknown mark.

Another function of organelle division is probably the regulation of organelle morphology, which can have a substantial physiological impact. For example, chloroplast division may be important in part because it generates smaller chloroplasts that can more readily redistribute in plant cells in response to changes in light intensity (*89*). However, the signaling processes and sensing mechanisms governing organelle division are completely unknown, although strong evidence exists that such signals are integrated with those controlling chloroplast expansion in plants (*62*) and mitochondrial fusion in yeast and animals (*10, 22, 90*) such that overall organelle morphology and volume are tightly regulated.

Recently, it was reported that mitochondrial fission occurs during apoptosis and is required for the release of cytochrome c from mitochondria and downstream cell death events (*4*). Cytological studies indicate that the Bcl-2 proapoptotic mitochondrial associated protein Bax colocalizes with assembled Drp1 structures on mitochondrial membranes, which implies that Bax interacts with the division machinery to promote apoptosis (*4*). These observations suggest that we are just beginning to understand the many functions associated with the division of organelles in eukaryotes.

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Cell Migration: Integrating Signals from Front to Back

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Cell migration is a highly integrated multistep process that orchestrates embryonic morphogenesis; contributes to tissue repair and regeneration; and drives disease progression in cancer, mental retardation, atherosclerosis, and arthritis. The migrating cell is highly polarized with complex regulatory pathways that spatially and temporally integrate its component processes. This review describes the mechanisms underlying the major steps of migration and the signaling pathways that regulate them, and outlines recent advances investigating the nature of polarity in migrating cells and the pathways that establish it.

Our liaison with cell migration, as humans, begins shortly after conception, accompanies us throughout life, and often contributes to our death. Although migratory phenomena are apparent as early as implantation, cell migration orchestrates morphogenesis throughout embryonic development (*1*). During gastrulation, for example, large groups of cells migrate collectively as sheets to form the resulting three-layer embryo. Subsequently, cells migrate from various epithelial layers to target locations, where they then differentiate to form the specialized cells that make up different tissues and organs. Analogous migrations occur in the adult. In the renewal of skin and intestine, fresh epithelial cells migrate up from the basal layer and the crypts, respectively. Migration is also a prominent component of tissue repair and immune surveillance, in which leukocytes from the circulation migrate into the surrounding tissue to destroy invading microorganisms and infected cells and to clear debris. The importance of cell migration however, goes far beyond humans and extends to plants and even to single-celled organisms (*2*).

Migration contributes to several important pathological processes, including vascular disease, osteoporosis, chronic inflammatory diseases such as rheumatoid arthritis and multiple

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