Mitotic Phosphorylation of Dynamin-related GTPase Drp1 Participates in Mitochondrial Fission*

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Mitochondria are double membrane organelles changing their size and position in their dynamic movement (1–6). Mitochondrial morphology varies in response to the environment and cellular differentiation; mitochondria form connected and filamentous network structures in most fibroblasts, are arranged along the myofibrils in skeletal muscle, and are coiled around the flagella in sperm. Mitochondrial morphology is maintained in a dynamic balance between fusion and fission (7–10). Mitofusin (Mfn, in mammals) and Fzo1 (in yeast) proteins are mitochondrial outer membrane GTPases that are essential for mitochondrial fusion (11–14). The dynamin-related GTPase OPA1 and a yeast homolog Mgm1, localized in the mitochondrial intermembrane space, are also required for fusion (15–17). These mitochondrial fusion proteins have important roles in mitochondrial function, as respiration is defective in fzo1- or mgm1-mutated yeast cells and Mfn- or OPA1-deficient mouse embryonic fibroblasts (12, 15, 17). The fusion proteins are also crucial for neuronal development, because Mfn2 and OPA1 are causal gene products of neuropathy, such as Charcot Marie tooth neuropathy type 2A and dominant optic atrophy type 1, respectively (18–20). Other dynamin-related proteins, Drp1 (mammals) and Dnm1 (yeast), participate in mitochondrial fission, and their mutation leads to highly elongated filamentous mitochondria structures (8, 21, 22). The partner proteins, mitochondrial outer membrane Fis1 and peripheral Mdv1 and CaF4, interact with Drp1/Dnm1 on the mitochondrial surface, although the homologs of WD motif proteins Mdv1 and CaF4 have not been found in mammalian cells (23–28). Dissipation of the mitochondrial membrane potential and apoptotic stimuli induce mitochondrial fragmentation by inhibiting fusion or stimulating fission (10, 29). The molecular mechanisms of the regulation of mitochondrial fusion and fission, however, are not well understood.

Mitochondria proliferate by growth and division of pre-existing mitochondria (30). The inheritance of mitochondria is well investigated in budding yeast by genetic and morphologic studies (6, 31). Yeast mitochondria form a network of interconnected tubules along actin filaments, and mitochondrial tubules are transported into the growing daughter buds (31). The mitochondrial movement depends on actin cables, class V myosin, Rab family GTPase Ypt11, and mitochondrial surface protein Mmr1 (31, 32). Mmm1, Mdm10, and Mdm12 were identified from the mitochondrial inheritance mutants, and they form a complex on the mitochondrial outer membrane (6). Mitochondrial filamentous structures are maintained through the mitotic stages, and long mitochondria are transported to the daughter buds. None of the known mitochondrial fission factors, Dnm1, Fis1, Mdv1, nor CaF4, is essential for mitochondrial inheritance or cell viability (6, 8, 22, 25). On the other hand, mitochondrial fragmentation is observed at specific stages in meiosis and sporulation (33). Mutant cells deficient in mitochondrial fission affect the uniform distribution of mitochondria into spores, which results in an increased number of inviable spores, although mitochondrial fission is not required for spore formation itself. It is less clear, however, how the interconnected mitochondrial network structures are inherited to mammalian daughter cells.

In this study, we analyzed mitochondrial dynamics and inheritance in mitotic mammalian cells. The interconnected mitochondrial network structures in interphase HeLa cells became fragmented in the early mitotic phase and stochastically segregated into two daughter cells. Finally, the filamentous mitochondria reformed in the daughter cells. The mitotic fragmentation required the mitochondrial fission factor Drp1. Furthermore, Drp1 was specifically phosphorylated by mitosis-
promoting factor (MPF, Cdk1/cyclin B), which stimulated mitotic mitochondrial fragmentation. The results demonstrated that mitochondrial morphology is regulated by Drp1-dependent mitochondrial fission in mitotic cells.

EXPERIMENTAL PROCEDURES

Materials—Monoclonal antibodies against FLAG (M2, Sigma), Drp1 (D80320, Transduction Laboratories, San Jose, CA), and cyclin B1 (sc-245, Santa Cruz Biotechnology, Santa Cruz, CA) were purchased from the indicated companies. Rabbit polyclonal antibodies against phosphorylated Drp1 were prepared using phosphorylated synthetic peptide IPIM-PASPQKHAVC (the underlined Ser residue was phosphorylated). Purified Cdk1/cyclin B was purchased from New England Biochemical (Beverly, MA) (P60205). Calf alkaline phosphatase was purchased from New England Biochemical (M0290S). Protein kinase A inhibitor was purchased from Sigma (P5990).

Plasmids—The mammalian su9-RFP expression plasmid has been described previously (10). The YFP*-a-tubulin expression plasmid was a generous gift from K. Ohashi and K. Mizuno, Tohoku University. The cDNA (encoding 705 amino acid residues) of rat Drp1 (10) was subcloned into pEGFP-N1 (for Drp1-GFP), p3xFLAG-CMV-10 (for FLAG-Drp1), or pET28a (for His-Drp1), respectively. All rat Drp1 mutants were prepared by PCR.

Cell Culture, Synchronization, Transfection, and Analysis—HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum under 5% CO2 at 37 °C. DNA transfection was performed using Lipofectamine, treated with thymidine again for 6–8 h. Mitotic Mitochondrial Fission by Phosphorylated Drp1

Mitochondria in the top fraction were traced, and the size distribution (in μm; 0–1, 1–2, 2–3, 3–4, 4–5, and longer than 5) was quantified using Metamorph software (Roper) and shown as a percentage (Figs. 2E and 5F).

RNA Interference (RNAi)—For human Drp1 RNAi, 27-base nucleotides were chemically synthesized (5'-ACAUUUGAGGAACUGCAAAUAAdAdG-3' and 5'-UAUAUUG-CAGUUCCUAAAGU-dAdT-3'). The annealed small interfering RNA (siRNA) was transfected to HeLa cells three times using Oligofectamine (Invitrogen) as described previously (26). Note that the target sequence is specific for human Drp1 but not for rat Drp1.

Protein Kinase Assay—Recombinant His-Drp1 proteins were expressed and purified by metal-chelating resin and further purified by ion exchange chromatography using Q-Fast Flow (Pharmacia, Uppsala, Sweden). The in vitro protein kinase reaction was analyzed as reported previously (34). Cdk1/cyclin B was isolated from mitotic HeLa cells as follows. Synchronized mitotic HeLa cells were solubilized by lysis buffer (40 mM Hepes-KOH buffer, pH 7.4, containing 60 mM β-glycerophosphate, 20 mM p-nitrophenylphosphate, 0.5 mM Na3VO4, 250 mM NaCl, 15 mM MgCl2, 1% Triton X-100, 5 mM dithiothreitol, and protease inhibitor mixture), and the lysate was incubated with antibody cyclin B1 and protein G-Sepharose, washed in lysis buffer, and then suspended in protein kinase buffer (20 mM Hepes-KOH buffer, pH 7.4, 15 mM EGTA, and 20 mM MgCl2).

Mitochondrial Dynamics in Mitotic HeLa Cells—Mitochondrial morphology is maintained under dynamic movement with a balance between fusion and fission. In most cultured fibroblasts, filamentous mitochondria form network structures in the cytoplasm. It is not well understood, however, how mammalian daughter cells inherit the interconnected mitochondria. Here we observed mitochondrial dynamics in mitotic HeLa cells (Fig. 1). The HeLa cells were synchronized at the G1/S phase using the double thymidine block procedure and subsequently released into thymidine-free medium to restart the cell cycle progression. Mitochondrial morphology was visualized using mitochondria-targeted DsRed (su9-RFP) in which mitochondrial-targeting sequence (1–69 residue segment) of Neurospora crassa F1-F1-ATPase subunit 9 was fused to the N-terminal end of DsRed.

First, we observed live images of mitochondrial dynamics in dividing cells using time-lapse video microscopy (supplemental Fig. S1A). The filamentous network structures of the mitochondria in the interphase cells changed to smaller fragmented structures in the early mitotic phase (supplemental Fig. S1A, a and b). Restoration of the filamentous structures began in the late mitotic phase (supplemental Fig. S1A, c and d). The mitochondria transmitted to the daughter cells regained normal filamentous network structures.
For further detailed analysis of the mitotic stages, the synchronized cells were fixed and counterstained with DAPI (Fig. 1A). The filamentous network structures of the mitochondria (tubular) were observed in most of the interphase cells (75% of counted cells) (Fig. 1A, p). From prophase to anaphase (Fig. 1A, q–s), <20% of the cells had filamentous mitochondria, and the number of cells with predominantly fragmented mitochondria clearly increased (~30%). About half of the early mitotic cells had shorter mitochondria compared with most of the mitochondria in the interphase cells (Fig. 1A, intermediate). In the late stages of mitosis (telophase and cytokinesis phases), there were more cells with tubular mitochondria (~50%) (Fig. 1A, t). These results indicated that mitochondrial morphology was drastically changed in HeLa cells, and fragmentation of mitochondria occurred during the early mitotic phase.

Thereafter, we analyzed statistically the size distribution of mitochondria in the mitotic cells. For this purpose, serial z-section images were obtained (from top to bottom) of the cells by confocal microscopy (supplemental Movies 1–5). The section images within the top one-fourth, middle one-fourth, or bottom one-fourth of the cell depth were compiled (top, middle, and bottom, respectively) and projected (Fig. 1B). In metaphase and anaphase, mitochondria were almost completely fragmented (morphometric quantification shown in Fig. 2E); images of the bottom fractions clearly show mitochondrial fragmentation (Fig. 1B), thus providing further support for the above conclusion.

Mitochondrial Fragmentation in Mitotic Cells Depends on Drp1—Next, we focused on the function of the mitochondrial fission factor Drp1 in mitotic mitochondrial fragmentation (Fig. 2). The expression of dominant negative mutant Drp1K38A inhibited mitochondrial fission and resulted in mitochn-
FIGURE 2. Inactivation of Drp1 induces morphologic changes of the mitochondria in the mitotic phase. A, knockdown of endogenous Drp1. siRNA specific for Drp1 or GFP (control) was transfected to HeLa cells. Total cell extracts were subjected to SDS-PAGE and subsequent immunoblot analysis using the indicated antibodies. TOM40, a mitochondrial protein as a loading marker. B, GFP (control) or Drp1-specific siRNA was transfected to HeLa cells and synchronized to M phase as described in the legend to Fig. 1A. The percentage of cells with the indicated mitochondrial morphology was counted as described in the legend to Fig. 1A. Control RNAi (gray bar) and Drp1 RNAi (black bar) cells in each stage (n > 100 of three independent experiments) were counted. C, Drp1 siRNA was transfected to HeLa cells, and mitochondrial morphology in each stage was analyzed by fluorescence microscopy as described in the legend to Fig. 1A. D, GFP (control) or Drp1-specific siRNA was transfected to HeLa cells, which were synchronized to the M phase and analyzed by confocal microscopy as in Fig. 1B. Compiled confocal images in cell periphery fractions are shown. E, quantification of mitochondrial fragmentation in the mitotic cells. GFP (control) or Drp1 siRNA was transfected to HeLa cells, which were synchronized to the M phase and analyzed by confocal microscopy as described for D. The mitochondrial length in peripheral fractions was analyzed by Metamorph software as described under “Experimental Procedures.” Control RNAi is as follows: interphase, n = 1070 (4 cells); prophase, n = 696 (4 cells); metaphase, n = 461 (3 cells); anaphase, n = 952 (8 cells); telophase, n = 542 (3 cells). Drp1 RNAi is as follows: interphase, n = 1156 (11 cells); prophase, n = 696 (4 cells); metaphase, n = 333 (4 cells); anaphase, n = 597 (6 cells); telophase, n = 525 (5 cells).
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—The antibody against Drp1 recognized at least three bands due to alternative splicing (Fig. 3A) (21). Here, these Drp1 bands in mitotic cells had lower gel mobility compared with those from G1/S cells (Fig. 3A). Exogenously expressed FLAG-tagged Drp1, detected as a single band in G1/S cells, was also shifted upward in mitotic cells (Fig. 4B, wt). All three bands of endogenous Drp1 proteins immunoisolated from mitotic cells were shifted down by treatment with alkaline phosphatase (Fig. 3A), suggesting that Drp1 is phosphorylated in mitosis.

To determine the protein kinase responsible for Drp1 phosphorylation, we used an in vitro phosphorylation assay (Fig. 3B). Cdk1/cyclin B (MPF) was immunoisolated from the mitotic HeLa cells using antibodies against cyclin B (34), and we confirmed that the isolated Cdk1/cyclin B had histone H1 kinase activity (data not shown). We used the immunoisolated Cdk1/cyclin B for phosphorylation of recombinant Drp1 with [γ-32P]ATP. Recombinant Drp1 was phosphorylated by the isolated Cdk1/cyclin B, as analyzed by autoradiography, and the band shift was also observed by Coomassie Brilliant Blue staining, suggesting that Drp1 is a substrate of Cdk1/cyclin B (Fig. 3B). To confirm whether Cdk1/cyclin B directly phosphorylates Drp1, we examined the protein kinase reaction using purified recombinant Cdk1/cyclin B (Fig. 3C). Drp1 was also phosphorylated by purified Cdk1/cyclin B, indicating that it acted as a responsible kinase of Drp1.

Serine 585 of Rat Drp1 Is Phosphorylated by Cdk1/cyclin B—Cdk1/cyclin B is a Ser/Thr protein kinase that recognizes the consensus motif (Ser/Thr)-Pro-Xaa-(Arg/Lys) of substrates; Arg/Lys at the +3 position is preferred but not essential for protein kinase recognition (35, 36). Rat Drp1 has four potential recognition sites for Cdk1/cyclin B, Ser-71, -126, -136, and -585; therefore, we constructed four Ser→Ala mutants, S71A, S126A, S136A, and S585A. The FLAG-tagged Drp1 mutant proteins were expressed in HeLa cells and synchronized at the mitotic phase (Fig. 4B). Immunoblots using anti-FLAG antibody revealed that the three Drp1 mutants, S71A, S126A, and S136A, as well as wild-type Drp1, were shifted upward, suggesting that these mutants were normally phosphorylated. In contrast, the S585A mutant in mitotic cells was detected as a single band with mobility similar to that of wild-type Drp1 in the G1/S phase, indicating that the phosphorylation at Ser-585 was abolished by this mutation. Recombinant Drp1 wt and the Ser→Ala mutants, except for Drp1S585A, were phosphorylated in vitro by immunoisolated or recombinant Cdk1/cyclin B (Fig. 4, C–E), indicating that Drp1 was mitotically phosphorylated on Ser-585 by Cdk1/cyclin B.

Driald elongation (10, 21). In live images, filamentous mitochondrial structures were maintained throughout mitosis in Drp1<sup>383K</sup>-expressing cells (supplemental Fig. S1B, a–h), suggesting that Drp1 activity was crucial for mitochondrial fragmentation during mitosis. In these cells, the mitochondria accumulated near the pole region but were still transmitted to the daughter cells (supplemental Fig. S1B, b–h). We further examined the effect of Drp1 knockdown using specific siRNA (Fig. 2). In control RNAi cells, mitochondria were fragmented in the early mitotic phase (~50%) (Fig. 2B and supplemental Fig. 1C, q and r), as in Fig. 1. In contrast, the elongated mitochondrial structure was maintained throughout mitosis in Drp1 knockdown cells (~80%) (Fig. 2, B and C, q and r). These results suggest that, in mitosis, mitochondria are fragmented by a Drp1-dependent fission reaction.

For statistic analysis of mitochondrial fragmentation, the length of mitochondria in mitotic cells was measured morphometrically as shown in Fig. 1B. Typical compiled images of the peripheral fraction are shown in Fig. 2D, and quantified data are shown in Fig. 2E. In control RNAi cells, the number of mitochondria <1 μm (~5% in interphase) clearly increased in mitosis (~30% in prophase and metaphase), and oppositely, the number of mitochondria longer than 4 μm (~30% in interphase) markedly decreased in the prophase and metaphase. On the other hand, in Drp1-RNAi cells, the formation of small mitochondrial fragments was clearly indicated that Drp1 is a substrate of Cdk1/cyclin B (Fig. 1). To confirm whether Cdk1/cyclin B directly phosphorylates Drp1, we used the immunoisolated Cdk1/cyclin B (34), and we confirmed that the isolated Cdk1/cyclin B had histone H1 kinase activity (data not shown). We used the immunoisolated Cdk1/cyclin B for phosphorylation of recombinant Drp1 with [γ-32P]ATP. Recombinant Drp1 was phosphorylated by the isolated Cdk1/cyclin B, as analyzed by autoradiography, and the band shift was also observed by Coomassie Brilliant Blue staining, suggesting that Drp1 is a substrate of Cdk1/cyclin B (Fig. 3B). To confirm whether Cdk1/cyclin B directly phosphorylates Drp1, we examined the protein kinase reaction using purified recombinant Cdk1/cyclin B (Fig. 3C). Drp1 was also phosphorylated by purified Cdk1/cyclin B, indicating that it acted as a responsible kinase of Drp1.
To further confirm the mitotic phosphorylation of Drp1, the antibodies specifically recognizing phosphorylation at Ser-585 in Drp1 (anti-p-Drp1) were prepared by immunizing rabbits with phosphorylated synthetic peptide. Rabbit polyclonal antibodies against phosphorylated Drp1 at Ser-585 (anti-p-Drp1) were prepared using phosphorylated synthetic peptide (IPIMPAS*PQGHAVC, Ser residue was phosphorylated (S*) and added with Cys residue to the C terminus; dotted underline). B, FLAG-tagged Drp1wt, Drp1S71A, Drp1S126A, Drp1S136A, or Drp1S585A was transfected in HeLa cells. These cells were synchronized in the G1/S or M phase, and total cell extracts were analyzed by immunoblotting using anti-FLAG antibodies. C, purified recombinant Drp1wt, Drp1S71A, Drp1S126A, or Drp1S585A was used as the substrate and immunosolated Cdk1/cyclin B as the enzyme. CBB, Coomassie Brilliant Blue. E, protein kinase reactions were performed as described for Fig. 3C using purified recombinant His-Drp1wt and His-Drp1S585A as substrates and recombinant Cdk1/cyclin B as the enzyme. F, left, HeLa cells were synchronized to the indicated phase and immunoprecipitated using anti-Drp1 antibodies. Immunoprecipitated proteins were analyzed by immunoblotting using anti-phosphorylated Drp1 peptide (top panel) or anti-Drp1 (bottom panel) antibodies. G, phosphorylated Drp1 as described for F was quantified by LAS3000. Shown are the results of three independent experiments.

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Phosphorylation of Drp1 at Ser-585 Stimulates Mitochondrial Fission—The sequence alignment with dynamin suggested that Ser-585 of Drp1 was present in the region with homology to the GTPase effector domain. The GTPase effector
domain stimulates the intra- or intermolecular interaction of dynamin and stimulates GTP hydrolysis activity (37). To examine the effect of the S585A mutation on GTPase activity, the GTP hydrolysis activity of the recombinant Drp1 proteins was analyzed using [γ-32P]GTP. Drp1S585A mutant and wild-type Drp1 exhibited indistinguishable GTPase activity, suggesting that Ser-585 was not critical for the GTP hydrolysis activity of Drp1 (data not shown).

To investigate the effect of Drp1 phosphorylation, the phosphorylation-negative mutant Drp1S585A was expressed in HeLa

![Figure 5](image-url)
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cells. In the mitotic cells expressing Drp1S585A, mitochondrial fragmentation was significantly repressed compared with the cells expressing wild-type Drp1 (Fig. 5, A and B). As expected, the cells with filamentous mitochondria were increased upon expression of the Drp1S585A mutant. These results suggest that phosphorylation at Ser-585 stimulates mitochondrial fission in mitosis.

In this experiment, the effect of exogenously expressed Drp1 was examined in the presence of endogenous Drp1. We therefore examined the effect of Drp1S585A in Drp1-repressed cells. Human Drp1 was knocked down in HeLa cells by the specific siRNA as described in the legend to Fig. 2, and then rat Drp1WT or Drp1S585A was expressed (Fig. 5, C–F). As described above, the repression of endogenous Drp1 inhibited mitochondrial fragmentation in mitotic cells, and the tubular mitochondria were maintained throughout the mitotic phase (Fig. 2, B–E). Expression of Drp1WT restored the mitotic mitochondrial fragmentation as in control RNAi cells (Fig. 5, C and D, compared with Fig. 2B). Morphometric analysis revealed that the number of short (<2 μm in length) mitochondria in early and late mitotic cells reached ~80% and ~50%, respectively (Fig. 5F). In marked contrast, the mitochondrial fragmentation was clearly affected by exogenous expression of Drp1S585A; the fragmented mitochondria with <2 μm decreased to ~50% and ~20% in the early mitotic and late mitotic phases, respectively. (Fig. 5; C, D, and F). These results suggest that phosphorylation at Ser-585 in mitosis stimulates mitochondrial fission.

DISCUSSION

In mitosis, organelles are inherited to daughter cells beyond dynamic changes of the membrane structures (7, 38, 39). The nuclear envelope and Golgi apparatus are broken down in early mitotic phase, transmitted to the daughter cells, and then reconstructed at a late stage of mitosis. Factors involved in Golgi assembly, GRASP65 (40), GM130 (41), and p47 (42), are phosphorylated during mitosis and stimulate mitotic Golgi disassembly. The inactivation of GRASP65 blocks mitotic Golgi fragmentation as well as mitotic entry (40). Here we examined the mitochondrial dynamics in mitotic HeLa cells. In mitosis, mitochondria were fragmented in a Drp1-dependent manner and transmitted to daughter cells, and then regained their filamentous structures. Drp1 was specifically phosphorylated at Ser-585 by Cdk1/cyclin B, which stimulated the mitochondrial fission in mitosis. From these results, we concluded that mitochondrial morphology is regulated by Drp1-dependent mitochondrial fission in mitotic cells. To our knowledge this is the first demonstration for Drp1-dependent mitochondrial fragmentation in the early mitotic phase.

Previous studies have demonstrated that the filamentous mitochondria in the G1 phase become shorter after the S phase in fibroblasts (43) and osteosarcoma cells (44). The dynamics of mitochondrial morphology in mitosis, however, have not been analyzed sufficiently. In the present study, we used video microscopy to examine mitochondrial dynamics in mitotic HeLa cells. HeLa cells have elongated and connected mitochondrial network structures, and mitochondria are fragmented in the mitotic phase. As expected, Drp1 was required for the mitotic mitochondrial fragmentation. In yeast, filamentous mitochondria are maintained and distributed to the daughter buds in mitosis, and the yeast homolog Dnm1 is not essential for mitosis (8, 22). In mitotic HeLa cells, fragmented mitochondria are distributed throughout the cytoplasm and almost equally transmitted to the two daughter cells. When Drp1 is inactivated, however, the mitochondria are not transmitted to the daughter cells equally. It is possible that mitochondrial fragmentation is not a checkpoint in mitotic entry, which is different from the fragmentation of the Golgi apparatus. Drp1-mediated mitochondrial fragmentation might allow mitochondria to disperse randomly throughout the cytoplasm and help ensure equal delivery of the mitochondria to the two daughter cells.

Drp1 was phosphorylated in mitosis by Cdk1/cyclin B, one of the major protein kinases involved in mitosis (35). The phosphorylation of Drp1 by cdk1/cyclin B stimulates mitochondrial fission activity. The phosphorylation consensus motif is conserved among mammalian Drp1 proteins, but not in yeast Dnm1, consistent with the notion that the filamentous mitochondrial networks are maintained throughout mitosis in yeast cells (7, 31, 32). Although substitution with an Asp residue is usually used to mimic a phosphorylated Ser residue, we did not observe any visible differences by the expression of Drp1S585D compared with wild-type Drp1 in mitochondrial morphology or in GTP hydrolysis activity (data not shown). It remains unknown how phosphorylation stimulates the mitochondrial fission activity of Drp1. Dynamin is phosphorylated by the Cdk family protein Cdk5 at their C-terminal Pro-rich domain (45, 46). This phosphorylation affects the complex formation with their partner proteins containing amphiphysin I and endophilin I, which regulates endocytic activity in neuronal cells (46, 47). Although the C-terminal Pro-rich region is not conserved in Drp1, five Pro residues are present near the Drp1 phosphorylation site (see Fig. 4A). Furthermore, the endophilin family protein endophilin B1 acting downstream of Drp1 is targeted from the cytoplasm to the mitochondrial fission foci to be involved in the fission reaction (48). Together, it is possible that the Cdk1/cyclin B-dependent Drp1 phosphorylation stimulates the interaction with endophilin B1 or other unidentified partner protein(s). Further analysis of the physiologic significance of the Drp1 phosphorylation will lead to new insights into the regulation of mitochondrial fission in response to cellular signaling and differentiation. Finally, it should be noted that we and others have previously demonstrated that treatment of cultured cells with nocodazole does not induce mitochondrial fragmentation (10, 49), suggesting that mitochondrial fragmentation in the mitotic phase is independent of disassembly of cytoplasmic microtubules (Fig. 1A, b and c).

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