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Bax/Bak-Dependent Release of DDP/TIMM8a Promotes Drp1-Mediated Mitochondrial Fission and Mitoptosis during Programmed Cell Death

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Summary

Mitochondrial morphology within cells is controlled by precisely regulated rates of fusion and fission [1–4]. During programmed cell death (PCD), mitochondria undergo extensive fragmentation [5–7] and ultimately caspase-independent elimination through a process known as mitoptosis [8]. Though this increased fragmentation is due to increased fission through the recruitment of the dynamin-like GTPase Drp1 to mitochondria [9, 10], as well as to a block in mitochondrial fusion [11, 12], cellular mechanisms underlying these processes remain unclear. Here, we describe a mechanism for the increased mitochondrial Drp1 levels and subsequent stimulation of mitochondrial fission seen during PCD. We observed Bax/Bak-mediated release of DDP/TIMM8a, a mitochondrial intermembrane space (IMS) protein [13, 14], into the cytoplasm, where it binds to and promotes the mitochondrial redistribution of Drp1, a mediator of mitochondrial fission. Using both loss- and gain-of-function assays, we also demonstrate that the Drp1- and DDP/TIMM8a-dependent mitochondrial fragmentation observed during PCD is an important step in mitoptosis, which in turn is involved in caspase-independent cell death. Thus, following Bax/Bak-mediated mitochondrial outer membrane permeabilization (MOMP), IMS proteins released comprise not only apoptogenic factors such as cytochrome c involved in caspase activation [15, 16] but also DDP/TIMM8a, which activates Drp1-mediated fission to promote mitochondrial fragmentation and subsequently elimination during PCD.

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Results and Discussion

Mitochondrial Fragmentation Is Involved in Mitoptosis

Mitochondrial fragmentation occurs during PCD and requires Drp1-mediated fission [10, 17] but not caspase activation [18, 19], and we hypothesized that a specific program of fragmentation-dependent mitochondrial elimination may be activated during PCD. To test this, HeLa cells were transiently cotransfected with expression vectors for mito-YFP and either wild-type Drp1, dominant-negative $Drp1^{K38A}$, or vector alone. The next day, cell death was initiated using staurosporine (STS) in the presence of the broad caspase inhibitor zVAD-fmk to prevent apoptosis, and cells were observed for 9 hr (Figure 1A). In control cells, mitochondria fragmented and subsequently began to disappear. Interestingly, although in Drp1-overexpressing cells mitochondrial fragmentation and disappearance were accelerated, overexpression of dominant-negative $Drp1^{K38A}$ resulted in a fused, interconnected mitochondrial network [20], and mitochondria were neither fragmented nor eliminated (Figure 1A). These results suggest that Drp1-dependent mitochondrial fission is involved in the caspase-independent mitochondrial elimination dubbed mitoptosis [8, 18, 19].

To examine the effects of Drp1-mediated mitochondrial fission on mitoptosis further, we investigated HeLa cells engineered so that expression of Drp1 or $Drp1^{K38A}$ is induced by tetracycline [17]. Following induction of Drp1 or $Drp1^{K38A}$ expression, these and control cells were left untreated or else treated for 9 hr with STS or actinomycin D (ActD) in the presence of the caspase inhibitor zVAD-fmk. Alternatively, cells were treated with STS or ActD and zVAD-fmk as above, washed, and returned for 3 days to medium containing only the caspase inhibitor. Mitochondrial content in whole-cell extracts was assessed by immunoblotting using CoxIV, an inner mitochondrial membrane protein; actin was used as a cytoskeletal marker (Figure 1B). Following treatment with STS or ActD in the presence of zVAD-fmk for 9 hr, mitochondrial disappearance was barely detectable. However, after these cells were returned to medium containing only zVAD-fmk for 3 days, mitochondrial disappearance as assessed using CoxIV levels was prominent while actin levels were unaffected, in agreement with previous reports [18, 19]. Mitochondrial elimination was similarly observed when levels of other mitochondrial membrane proteins such as succinate-ubiquinol oxidoreductase or NADH oxidoreductase were evaluated (data not shown). Mitochondrial fragmentation and ultimately elimination were confirmed by electron

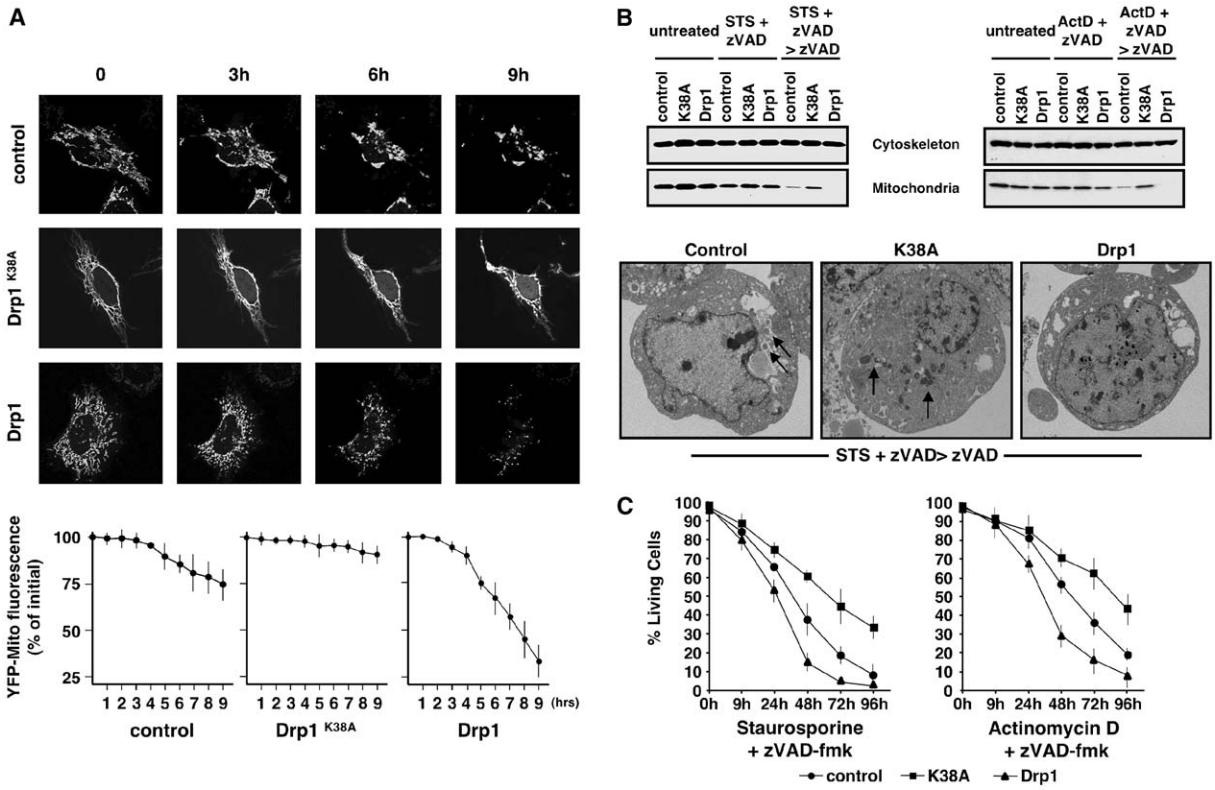

Figure 1. Mitochondrial Fragmentation Is Involved in Mitochondrial Elimination

(A) HeLa cells cotransfected with mito-YFP and either pcDNA3 (control), pcDNA3-Drp1^{K38A} (Drp1^{K38A}), or pcDNA3-Drp1 (Drp1) were treated with 1 μM STS in the presence of 100 μM zVAD-fmk, then images were acquired by confocal microscopy. Graphics show percent of total mito-YFP fluorescence remaining at the indicated times as assessed using MetaMorph software. Data represent the means ±SD of 13 single-cell time-lapse measurements per time point.

(B) T-Rex-V (control), T-Rex-Drp1 (Drp1), and T-Rex-Drp1^{K38A} (K38A) HeLa cells [17] were pretreated with tetracycline to induce Drp1 and Drp1^{K38A} expression, then either left untreated or else treated for 9 hr with 1 μM STS or 10 μM ActD in the presence of zVAD-fmk (STS + zVAD, ActD + zVAD). Alternatively, cells were treated with 1 μM STS or 10 μM ActD as well as zVAD-fmk for 9 hr, washed, and returned to medium containing only zVAD-fmk (STS + zVAD > zVAD or ActD + zVAD > zVAD) for 3 days. Levels of mitochondrial and cytoskeletal markers in total cell extracts were assessed by immunoblotting using anti-CoxIV and anti-actin antibodies, respectively (upper panel). Mitochondrial disappearance after 3 days (STS + zVAD > zVAD) was investigated by transmission electron microscopy (lower panel). In control cells, arrows identify fragmented mitochondria within an autophagosome, and, in the Drp1^{K38A}-expressing cells, arrows indicate mitochondria. In Drp1-overexpressing cells, mitochondria are not seen, having undergone elimination.

(C) After tetracycline pretreatment, T-Rex-V (control), T-Rex-Drp1 (Drp1), and T-Rex-Drp1^{K38A} (K38A) HeLa cells were treated for 9 hr with 1 μM STS or 10 μM ActD in the presence of zVAD-fmk and then with new medium containing only zVAD-fmk, changed every 24 hr. Cell viability was assessed by trypan blue exclusion at the indicated times. Data represent the means ±SD of four independent experiments, 300 cells per condition.

microscopy and, interestingly, fragmented mitochondria were found within autophagosomes (Figure 1B). Importantly, mitochondrial disappearance was increased in Drp1-expressing cells but inhibited in Drp1^{K38A}-expressing cells (Figure 1B), further demonstrating that mitochondrial fragmentation depends on Drp1 and is involved in caspase-independent mitoptosis.

Cell death can occur even in the absence of caspase activation [21, 22], and we investigated whether mitoptosis might be involved in caspase-independent cell death. Control or else Drp1- or Drp1^{K38A}-expressing cells were incubated for 9 hr with STS or ActD in the presence of zVAD-fmk, then washed and returned to media containing only zVAD-fmk. The percentage of living cells was assessed periodically, and each day, cells were incubated in fresh media containing zVAD-fmk. We observed greater caspase-independent cell death in Drp1-expressing cells than in controls, while Drp1^{K38A}-expressing cells were more resistant (Figure 1C). Involvement of mitoptosis in caspase-independent cell death was similarly demonstrated using cells in which caspase-9 expression was suppressed using siRNA (Figure S1). Thus, our results indicate that mitoptosis involving Drp1-dependent mitochondrial fragmentation participates in caspase-independent cell death.

Drp1 Binds DDP/TIMM8a, an IMS Protein Released from Mitochondria during PCD

It is generally accepted that PCD occurs downstream of Bax/Bak-mediated MOMP [15, 23], and we have observed that Bcl-2 family members regulate mitochondrial fragmentation and subsequent mitoptosis during PCD (Figure S2). Since Drp1-mediated fission is involved in mitochondrial fragmentation during PCD [10] and Bax overexpression increases mitochondrial fragmentation (Figure S2A), we considered the possibility

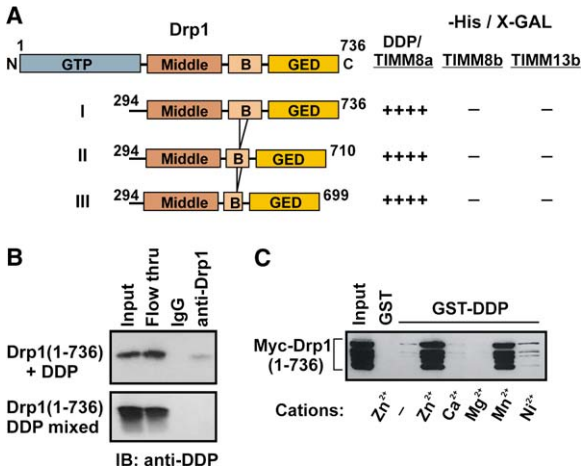

Figure 2. The Mitochondrial IMS Protein DDP/TIMM8a Interacts with Drp1

(A) Yeast two-hybrid analyses of the DDP-Drp1 interaction. pBHA-DDP/TIMM8a or the related baits TIMM8b or TIMM13b were tested against the indicated pGAD10-Drp1 constructs. Drp1 domains are indicated schematically: GTP, GTP binding domain; M, middle domain; B, insert B; GED, α-helical GTPase effector domain. Strength of interaction was assayed by β-gal and *HIS3* induction as described previously [33]. I, II, and III depict the known Drp1 splice variants, with deletions within the B insert of variants II and III shown schematically.

(B) Extracts from COS-7 cells coexpressing Myc-Drp1(1–736) and DDP were immunoprecipitated with anti-Drp1 antibodies and immunoblotted for DDP. In control experiments, extracts from DDP-transfected cells were mixed with extracts from Myc-Drp1-transfected cells, then immunoprecipitated as above. "Input" represents 20% of the starting material, "flow thru" 20% of the supernatant from the anti-Drp1 immunoprecipitation, and "anti-Drp1" the immunoprecipitate. "IgG" represents an immunoprecipitation done with control nonimmune IgG.

(C) GST-DDP bound to glutathione beads was incubated in vitro with extracts from cells overexpressing Myc-Drp1(1–736). Divalent cations were added at 1 mM for Ca^{2+}, Mg^{2+}, Mn^{2+}, and Ni^{2+} and 200 μM for Zn^{2+}. Bound proteins were resolved by SDS-PAGE and immunoblotted with anti-Myc antibodies. Multiple bands observed for Drp1(1–736) likely reflect protein degradation, as these experiments were conducted in the absence of metal chelators or protease inhibitors.

that Bax might also regulate Drp1 function. Since we and others have observed that mitochondrial fragmentation largely occurs downstream of Bax/Bak-mediated MOMP [11, 24, 25], we hypothesized that Bax/Bak may activate Drp1 indirectly via release of a mitochondrial factor, reminiscent of the Bax/Bak-mediated triggering of caspase activation through the release of cytochrome c [15].

Multiple yeast two-hybrid screens were performed using Drp1 as well as several mitochondrial IMS proteins as baits, searching for novel interactions. In a screen using the IMS protein DDP/TIMM8a as bait, an interaction was found with a fragment of Drp1 (variant I) comprising amino acid residues 294–736 (Figure 2A). All six strongly positive clones corresponded to this fragment, and thus DDP represented a compelling candidate for activation and/or mitochondrial recruitment of Drp1. DDP is similar in structure to several small yeast Tim and mammalian TIMM proteins, all harboring a conserved "twin Cx_3C" paired dicysteine motif, that are implicated in the transport of transmembrane carrier proteins across the aqueous IMS to the inner mitochondrial membrane [13, 14, 26]. However, yeast two-hybrid tests using human TIMM proteins such as the DDP/TIMM8a paralog TIMM8b (like DDP, orthologous to yeast Tim8) and TIMM13b [13, 14] yielded no interactions with Drp1, demonstrating the selectivity of the DDP-Drp1 interaction. Drp1 exists in three known splice variants (I–III), and all interact robustly with DDP (Figure 2A).

To confirm the DDP-Drp1 interaction in cells, we performed coimmunoprecipitation studies. In cells overexpressing full-length Drp1(1–736) together with DDP, anti-Drp1 antibodies coprecipitate a small fraction of DDP (Figure 2B). In control experiments using postlysis mixtures of detergent extracts from cells individually expressing DDP or Drp1(1–736), no coimmunoprecipitation of DDP and Drp1 is observed, and immunoprecipitations using nonspecific IgG similarly revealed no precipitation of DDP or Drp1 (Figure 2B; data not shown). Normally, DDP is localized to the mitochondrial IMS, while Drp1 is predominantly cytoplasmic. However, when overexpressed, a small portion of DDP is cytoplasmic (data not shown; [27]), and thus the interaction with Drp1 may be occurring there. Lastly, many known interactions of the small IMS Tim proteins are stimulated by Zn^{2+} [28–33]. Using glutathione S-transferase (GST) "pull-down" experiments, we similarly found that Drp1 binding to DDP in vitro was enhanced markedly by the addition of Zn^{2+} or Mn^{2+} but not by Ca^{2+} or Mg^{2+} (Figure 2C).

We narrowed down the interaction domains within both Drp1 and DDP required for the DDP-Drp1 interaction. Deletion analyses of DDP revealed that the N-terminal 17 and C-terminal 9 amino acids of DDP are not required for strong interaction with Drp1. However, a central region of DDP containing the conserved "twin Cx_3C" motif (residues 35–74) bound very weakly to Drp1 (Figure S3A). We generated a DDP-TIMM8b chimera by replacing amino acid residues 59–97 of DDP with the corresponding residues 52–83 of its shorter paralog TIMM8b, retaining the conserved twin Cx_3C motif. The DDP-TIMM8b chimera did not interact with Drp1(294–736), indicating that residues in the C-terminal third of DDP are critical for the interaction (Figure S3A).

We attempted to narrow down the interaction domain within Drp1 required for DDP interaction using yeast two-hybrid tests. A fragment comprising Drp1 residues 303–736 interacted robustly with DDP; however, Drp1(316–736) exhibited a much weaker interaction, and Drp1(1–489) did not interact at all (data not shown). Thus, we pursued other techniques. Using a nuclear redistribution assay [33], coimmunoprecipitation experiments, and GST pull-downs, we found that Drp1(502–736) was sufficient for strong interaction with DDP (Figures S3B–S3H). Thus, the interaction domain sufficient for strong DDP binding was narrowed to residues 502–736 of Drp1, which harbors the critical GTP-effector domain (GED) and B loop [34, 35] and is important for intra- and intermolecular Drp1 interactions [35]. Interestingly, it was recently reported that the GED domain of Drp1 alone targets specifically and exclusively to mitochondria, supporting its involvement in localizing Drp1 to mitochondria [36].

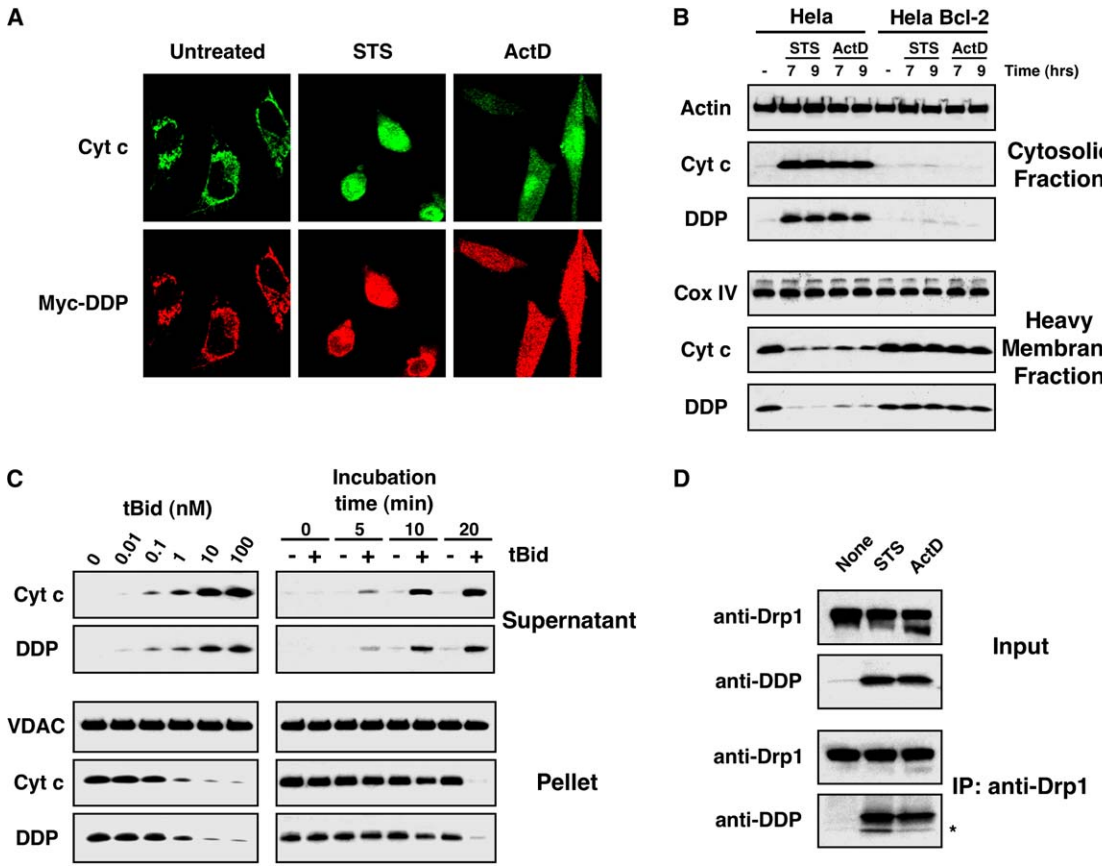

Figure 3. DDP/TIMM8a, a Protein Coreleased with Cytochrome c during PCD, Binds Drp1 in the Cytoplasm

(A) HeLa cells transfected with Myc-DDP were either left untreated or treated with 1 μM STS or 10 μM ActD for 8 hr, fixed, stained with anti-cytochrome c (green) and anti-Myc (red) antibodies, and analyzed by confocal microscopy.

(B) HeLa cells or HeLa cells stably overexpressing Bcl-2 were treated for 7 and 9 hr with 1 μM STS or 10 μM ActD. Next, the cytosolic and heavy membrane fractions were analyzed by immunoblotting for the presence of cytochrome c and DDP. As loading controls, actin was used for the cytosolic fraction and CoxIV for the heavy membrane fraction.

(C) Mitochondria isolated from HeLa cells were incubated (20 min, 30ºC) with different concentrations (nM) of recombinant tBid. Mitochondrial and supernatant fractions were resolved by SDS-PAGE and immunoblotted with antibodies against the indicated proteins. Mitochondria were also incubated with (+) or without (−) 10 nM tBid at 30ºC, and the mitochondrial pellet and supernatant were analyzed at different time points. Equal loading of the mitochondrial pellet lanes was monitored using anti-VDAC antibodies.

(D) Cytosolic fractions of untreated or else STS- (1 μM, 7 hr) or ActD- (10 μM, 7 hr) treated HeLa cells were immunoprecipitated using anti-Drp1 antibodies, and immunoblotted for DDP and Drp1. Immunoprecipitations using nonspecific IgG revealed no precipitation of DDP (data not shown). "Input" represents 10% of the starting material. An asterisk identifies a probable DDP breakdown fragment.

As mentioned previously, DDP is normally localized within the mitochondrial IMS, separated from cytoplasmic Drp1 by the mitochondrial outer membrane. However, one scenario for a physiological interaction between Drp1 and DDP occurs during PCD, when some IMS proteins are released into the cytoplasm after MOMP [15, 16]. Indeed, immunocytochemical and cell fractionation studies (Figures 3A–3B) confirm that DDP, like cytochrome c, is released during PCD [37] as a consequence of Bax/Bak-mediated MOMP, and its release is prevented by the antiapoptotic protein Bcl-2 (Figure 3B).

Mitochondria isolated from HeLa cells were incubated with recombinant tBid, a BH3-only member of the Bcl-2 family that requires either Bax or Bak to trigger MOMP [23]. Both dose-escalation and kinetic studies in vitro with tBid demonstrate that DDP and cytochrome c are coreleased after MOMP (Figure 3C). Studies utilizing embryonic fibroblasts from knockout mice lacking both Bax and Bak further confirmed that DDP requires Bax and/or Bak to be coreleased with cytochrome c during PCD (Figure S4). Since DDP was detected in the cytosol of apoptotic cells (Figure 3B), we investigated the interaction between DDP and Drp1 during PCD. By coimmunoprecipitation, a robust interaction was detected between DDP and Drp1 in the cytosol of apoptotic cells but not in nonapoptotic cells (Figure 3D). Thus, an interaction between DDP and Drp1 occurs prominently during PCD, prefiguring a role for released DDP in the regulation of Drp1 function.

DDP Release Participates in Drp1 Mitochondrial Recruitment/Retention and Subsequent Mitoptosis

Since Drp1 is recruited to and/or retained on mitochondria during PCD [9, 10] (Figure S2B), we studied whether DDP binding might play a role. HeLa cells

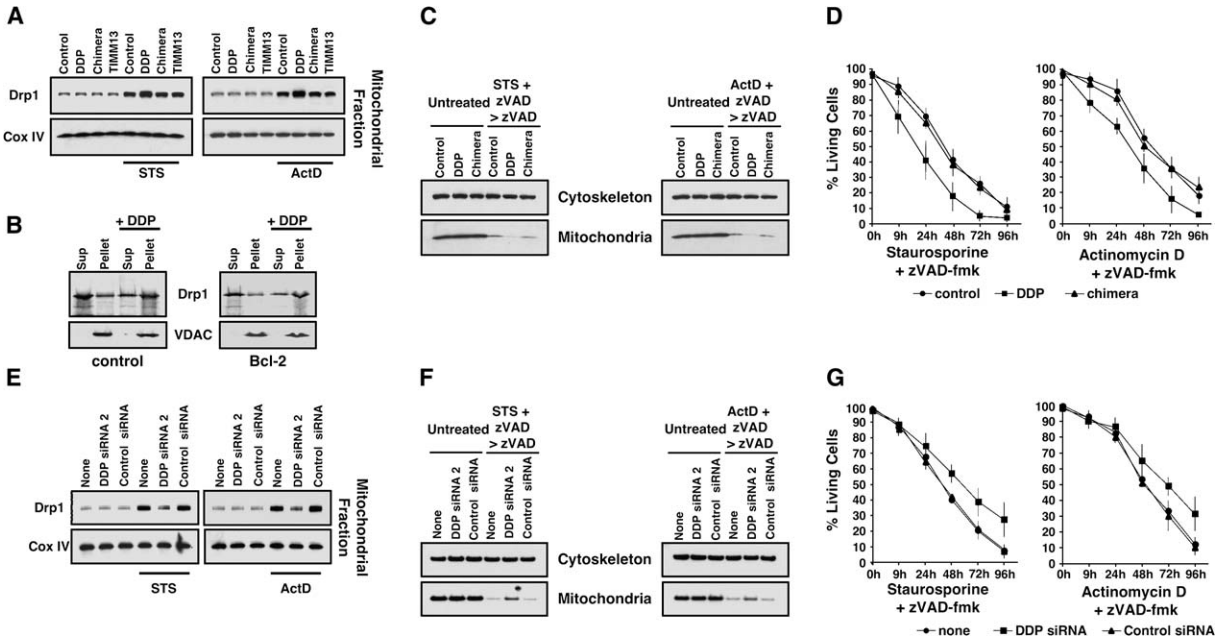

Figure 4. DDP Promotes Drp1 Mitochondrial Recruitment and Subsequent Mitoptosis during PCD

(A) HeLa cells transiently transfected with vector alone (Control), Myc-DDP (DDP), a Myc-DDP-TIMM8b chimera (Chimera), or Myc-TIMM13b (TIMM13) were either left untreated or treated 9 hr with 1 μM STS or 10 μM ActD in the presence of 100 μM zVAD-fmk. Levels of Drp1 in the mitochondrial fraction were assessed by immunoblotting, with CoxIV as a loading control. Expression of the Myc-tagged proteins was equal, as determined by immunoblotting with anti-Myc antibodies (data not shown).

(B) In vitro-translated [^{35}S]Drp1(294–736) was incubated with mitochondria isolated from either control or Bcl-2-overexpressing HeLa cells in the presence or absence of in vitro-translated [^{35}S]DDP. Levels of Drp1(294–736) were then evaluated in the supernatant (Sup) or mitochondrial pellet after SDS-PAGE. VDAC was used as a loading control for the mitochondrial pellet.

(C) HeLa cells transiently transfected with vector alone (Control), Myc-DDP (DDP), or a DDP-TIMM8b chimera (Chimera) were either left untreated or treated with 1 μM STS or 10 μM ActD with zVAD-fmk for 9 hr, washed, and returned for 3 days to medium containing only zVAD-fmk ([STS + zVAD > zVAD] or [ActD + zVAD > zVAD]). Mitochondrial and actin levels were assessed as in Figure 1B.

(D) HeLa cells were transfected and treated as in (C), and cell viability was assessed at the indicated times using trypan blue exclusion. Data represent means ±SD of three independent experiments, 300 cells per condition.

(E) HeLa cells were either left untransfected (none) or transfected with DDP siRNA 2 or control siRNA. Next, cells were either left untreated or treated 9 hr with 1 μM STS or 10 μM ActD in the presence of zVAD-fmk. Levels of Drp1 in the mitochondrial fraction were then assessed by immunoblotting. CoxIV was used as a loading control.

(F) HeLa cells were either not transfected (none) or transfected with DDP siRNA 2 or control siRNA. Cells were then either left untreated or treated with 1 μM STS or 10 μM ActD with zVAD-fmk for 9 hr, washed, and returned for 3 days to medium containing only zVAD-fmk [(STS + zVAD > zVAD) or (ActD + zVAD > zVAD)]. Mitochondrial and cytoskeletal protein levels were assessed as in (C).

(G) HeLa cells were either not transfected (none) or transfected with DDP siRNA 2 or control siRNA as in (F) and then treated and analyzed for viability using trypan blue exclusion. Data represent the mean ±SD of three independent experiments, 300 cells per condition.

were transfected with either vector alone or a Myc-DDP expression construct. As additional controls, we also used constructs encoding a Myc-DDP-TIMM8b chimera or Myc-TIMM13b, neither of which interacts with Drp1 (Figure 2A) but which, like Myc-DDP, localize to the IMS (data not shown). Transfected cells were either left untreated or else incubated with STS or ActD to induce MOMP, then fractionated. Mitochondrial Drp1 levels from Myc-DDP-overexpressing cells were increased after MOMP as compared to the levels in each of the different controls (Figure 4A), suggesting that DDP binding to Drp1 increases Drp1 recruitment to the mitochondria and/or its retention there.

To determine whether binding to DDP is involved in mitochondrial recruitment of Drp1, we employed an in vitro system, using isolated mitochondria incubated with in vitro-translated Drp1 and DDP. When full-length Drp1 was incubated with mitochondria, its association with mitochondria was barely detectable, even in the presence of DDP (data not shown), suggesting that either in vitro-translated full-length Drp1 was misfolded or else that full-length Drp1 must undergo a conformational change in vivo to facilitate its association with mitochondria. However, a fragment of Drp1 lacking most of the GTP binding domain but still capable of binding DDP in yeast two-hybrid tests, Drp1(294–736) (Figure 2A), associated spontaneously with mitochondria in vitro (Figure 4B). Interestingly, in the presence of DDP, the fraction of Drp1(294–736) in the mitochondrial pellet was increased (Figure 4B). In control experiments, the addition of in vitro-translated TIMM13b, which does not bind Drp1, had no effect on Drp1(294–736) recruitment (data not shown). These data suggest that Drp1(294–736) may mimic a Drp1 conformation competent for mitochondrial association and that the Drp1-DDP interaction may increase mitochondrial recruitment of Drp1 during PCD, though it remains possible that it promotes retention as well. Overexpression of Bcl-2 in cells prior to isolation of mitochondria had no effect on the cell-free mitochondrial association of

Drp1(294–736), either in the absence or presence of DDP (Figure 4B).

Consistent with the observation that DDP-overexpressing cells have higher mitochondrial levels of Drp1 than control cells during PCD, upon treatment with either STS or ActD we found that DDP-overexpressing cells also showed an increase in mitoptosis in comparison to control and DDP-TIMM8b chimera- or TIMM13b-transfected cells (Figure 4C; data not shown). In fact, DDP-transfected cells not only showed an increase in mitoptosis but were also more prone to caspase-independent cell death (Figure 4D).

To investigate further the involvement of DDP in Drp1 mitochondrial recruitment/retention as well as mitochondrial fragmentation during PCD, DDP expression was knocked down using siRNAs (Figure S5). Importantly, total cellular Drp1 levels were unchanged in DDP siRNA-transfected cells, and DDP siRNA did not affect apoptosis, mitochondrial morphology, or mitochondrial inner membrane cristae structure (Figure S5). Since our earlier results suggested that DDP, once released into the cytoplasm during PCD, binds to Drp1 and promotes its mitochondrial localization (Figure 4A), we anticipated that mitochondrial Drp1 levels would be decreased after cell death induction in cells lacking DDP, as compared with control cells. This is indeed what we observed (Figure 4E), confirming that DDP is a mediator of Drp1 mitochondrial localization during PCD.

Given that the siRNA-mediated knockdown of DDP decreases mitochondrial levels of Drp1, we asked whether knockdown of DDP also inhibits mitoptosis. Following treatment with either STS or ActD, caspase-independent mitoptosis occurs normally in untransfected or control siRNA-transfected cells but is significantly prevented in DDP siRNA-transfected cells (Figure 4F). Concordant with this observation, DDP siRNA-transfected cells appeared more resistant to caspase-independent cell death in comparison to untransfected or control siRNA-transfected cells (Figure 4G).

Conclusions

We report that increased mitochondrial fragmentation during PCD occurs partly as a result of increased Drp1-mediated mitochondrial fission through interaction of Drp1 with DDP/TIMM8a. We found that, after Bax/Bak-mediated MOMP, DDP is released from mitochondria into the cytoplasm, where it binds to the C-terminal portion of Drp1. This interaction increases Drp1 recruitment and/or retention to mitochondria, where Drp1 promotes mitochondrial fission and, subsequently, mitoptosis. Though the molecular mechanisms whereby DDP increases Drp1 mitochondrial levels during PCD remain unclear, DDP binding to Drp1 may cause changes in Drp1 oligomerization or increased affinity of Drp1 for another protein that mediates its mitochondrial localization. Alternatively, DDP may "chaperone" Drp1 directly to the mitochondrial surface.

Though our results regarding the timing of release of DDP and cytochrome c relative to mitochondrial fragmentation during PCD are consistent with a number of previous studies [11, 24, 25, 38], they appear at odds with some models of mitochondrial fragmentation during PCD [38]. Some investigators have suggested that fragmentation is required for release of apoptogenic factors from the IMS, since overexpression of dominant-negative Drp1^{K38A} results in long, fused mitochondria by inhibiting mitochondrial fission [10], and cytochrome c is not released during apoptosis [38]. However, we have found that DDP and other apoptogenic IMS proteins such as Smac/DIABLO and Omi/HtrA2 are released in the Drp1^{K38A}-expressing cells, indicating that MOMP has occurred (Figure S6; data not shown), while cytochrome c is still retained. Thus, the retention of cytochrome c under these conditions is likely due to mechanisms other than a general block of MOMP and does not necessarily reflect the behavior of other IMS proteins.

Lastly, many reports have described autophagic cell death as an alternative form of PCD [39, 40]. Interestingly, during autophagic cell death, fragmented mitochondria are found within autophagosomes (Figure 1B; [41, 42]). Since we report here that mitoptosis is initiated after Bax/Bak-mediated MOMP, autophagic cell death could also be a consequence of MOMP [41, 42], and DDP may play a role. Further studies of the regulation of Drp1 function during PCD will be important to clarify the physiological role of the DDP interaction in these processes.

Supplemental Data

Supplemental Data include six figures and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/15/23/2112/DC1/.

Acknowledgments

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