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YME1L degradation reduces mitochondrial proteolytic capacity during oxidative stress

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Abstract

Mitochondrial proteostasis is maintained by a network of ATP-dependent quality control proteases including the inner membrane protease YME1L. Here, we show that YME1L is a stress-sensitive mitochondrial protease that is rapidly degraded in response to acute oxidative stress. This degradation requires reductions in cellular ATP and involves the activity of the ATP-independent protease OMA1. Oxidative stress-dependent reductions in YME1L inhibit protective YME1L-dependent functions and increase cellular sensitivity to oxidative insult. Collectively, our results identify stress-induced YME1L degradation as a biologic process that attenuates protective regulation of mitochondrial proteostasis and promotes cellular death in response to oxidative stress.

Introduction

The mitochondrial inner membrane is the site of essential mitochondrial functions including electron transport chain activity, phospholipid metabolism, and the regulation of apoptotic signaling. Dysfunction of these processes is intricately associated with the onset and pathology of etiologically diverse human diseases including cancer, diabetes, and neurodegenerative diseases [1–5]. Despite the involvement of mitochondrial dysfunction in disease pathogenesis, the underlying molecular mechanisms that lead to altered mitochondrial function in these disorders remain poorly defined. One potential mechanism to explain alterations in mitochondrial function in disease is a decreased capacity to maintain mitochondrial inner membrane protein homeostasis (or proteostasis).

Mitochondrial inner membrane proteostasis is maintained by ATP-dependent AAA+ quality control proteases including YME1L, AFG3L2, and paraplegin. These proteases assemble into homo- or hetero-oligomeric complexes with active sites oriented toward the intermembrane space (IMS; YME1L homooligomers) or the mitochondrial matrix (AFG3L2 homooligomers and AFG3L2/paraplegin heterooligomers) (Fig 1A) [6]. Mitochondrial inner membrane proteases are involved in nearly all aspects of mitochondrial biology including the maintenance of electron transport chain integrity, regulation of phospholipid metabolism, and the control of mitochondrial morphology and dynamics [7]. Alterations in the activity of these proteases significantly impair mitochondrial functions. Genetic depletion of *YME1L* decreases cellular capacity to regulate mitochondrial inner membrane proteostasis, impairs the regulation of mitochondrial dynamics, alters phospholipid metabolism, attenuates the activity of the electron transport chain, and increases stress sensitivity to apoptotic stimuli [8–12]. Similarly, loss of AFG3L2 disrupts mitochondrial proteostasis, mitochondrial protein translation, and trafficking of mitochondria in neuronal axons [13–15]. Mutations in AFG3L2 and paraplegin are also associated with the neurodegenerative disorders spinocerebellar ataxia type 28 and hereditary spastic paraplegia type 7, respectively [16,17]. Thus, alterations in the activity of these proteases could disrupt mitochondrial inner membrane proteostasis and contribute to the pathologic mitochondrial dysfunction associated with human disease.

Here, we identify YME1L as a stress-sensitive mitochondrial inner membrane protease. We show that YME1L is degraded in response to acute oxidative stress in a mechanism involving reductions in cellular ATP and the activity of the ATP-independent mitochondrial inner membrane protease OMA1. Furthermore, we show that stress-induced YME1L degradation impairs YME1L-dependent regulation of mitochondrial inner membrane proteostasis, sensitizing cells to oxidative insult. Collectively, our work reveals stress-induced YME1L degradation as a mechanism that can contribute to the pathologic mitochondrial dysfunction associated with many human diseases.

Results and Discussion

YME1L is degraded in response to oxidative stress

In order to identify stress-induced alterations in inner membrane protease composition, we monitored YME1L and AFG3L2 protein levels by quantitative immunoblotting in cells treated with various oxidative stressors. H_2O_2 decreased YME1L and AFG3L2 protein levels in SHSY5Y cells (Fig 1B and C). Other mitochondrial inner membrane proteins such as Tim23 were not affected by H_2O_2
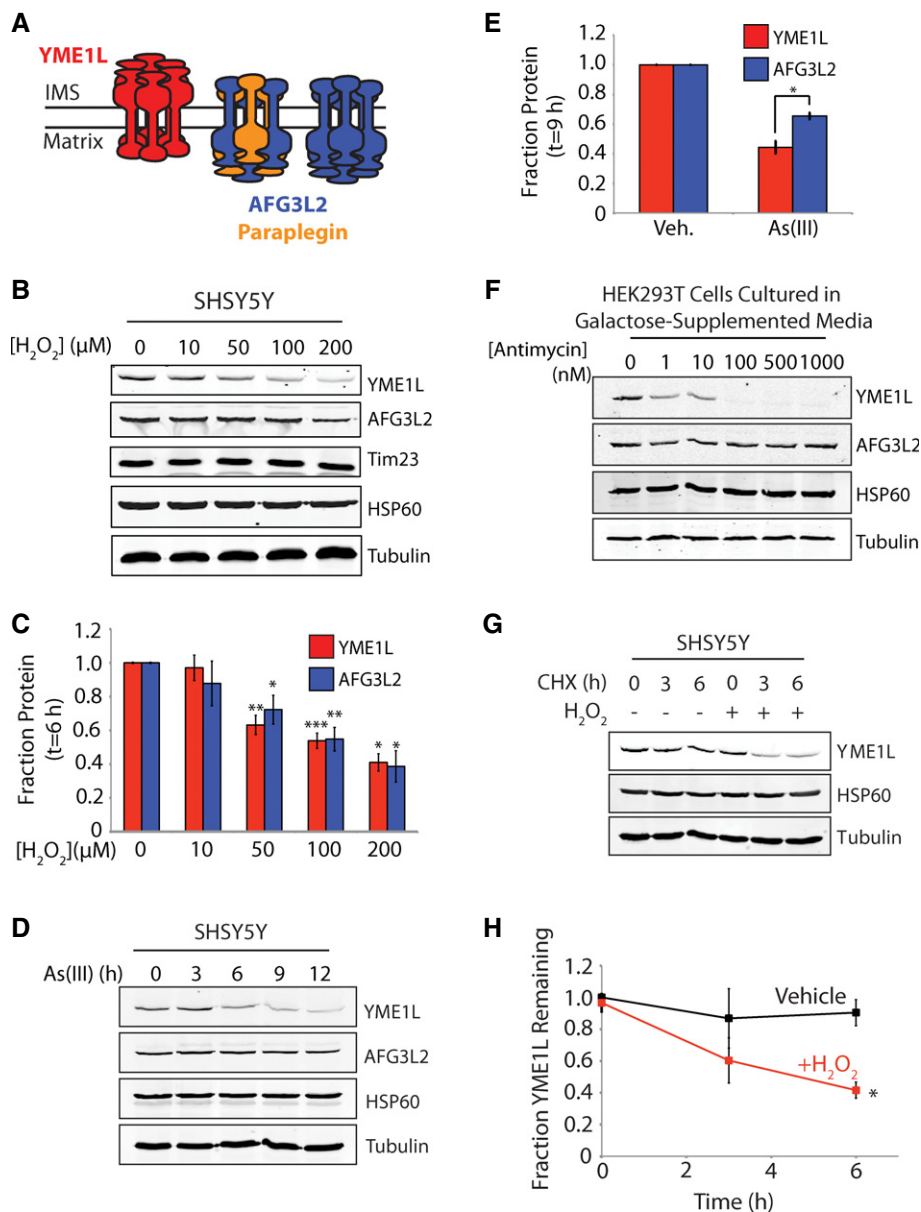
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Figure 1. YME1L is a stress-sensitive mitochondrial protease.

A   Illustration showing the oligomer assembly and active site orientation of YME1L (red), AFG3L2 (blue), and paraplegin (orange) in the mitochondrial inner membrane.

B   Representative immunoblot of lysates prepared from SHSY5Y cells treated with the indicated concentration of H_2O_2 for 6 h.

C   Quantification of YME1L and AFG3L2 from immunoblots as shown in (B). Error bars show SEM for $n \geq 3$. ***P-value < 0.001, **P-value < 0.01, *P-value < 0.05.

D   Representative immunoblot of lysates prepared from SHSY5Y cells treated with As(III) (50 μM) for the indicated time.

E   Quantification of YME1L and AFG3L2 in SHSY5Y cells treated with As(III) (50 μM; 9 h) from immunoblots as shown in (D). Error bars show SEM for $n = 4$. *P-value < 0.05.

F   Immunoblots of lysates prepared from HEK293T cells cultured in galactose-supplemented media treated with the indicated concentration of antimycin A for 6 h.

G   Representative immunoblot of lysates prepared from SHSY5Y cells treated as indicated with cycloheximide (CHX; 50 μg ml^{-1}) and H_2O_2 (100 μM) for the indicated time.

H   Quantification of YME1L protein levels from immunoblots as shown in (G). Error bars show SEM for $n = 3$. *P-value < 0.05.

Source data are available online for this figure.

(Fig 1B, Supplementary Fig S1A). Similar results were observed in all cell lines tested including N2a, HEK293T, and HeLa cells—the latter, a cell line that lacks *PARKIN* [18–20], indicating that the loss of YME1L and AFG3L2 is independent of mitophagy (Supplementary Fig S1B–D). The superoxide generator paraquat also reduced YME1L and AFG3L2 protein levels in SHSY5Y cells (Supplementary Fig S1E). Alternatively, the environmental toxin As(III) selectively reduced YME1L protein levels relative to AFG3L2 (Fig 1D and E).

YME1L was also substantially decreased in HEK293T cells cultured in galactose-supplemented media treated with the complex III inhibitor antimycin A, while AFG3L2 was not affected in these cells (Fig 1F). The addition of antimycin A to cells cultured in glucose-containing media did not influence YME1L protein levels (Supplementary Fig S1F). These results show that YME1L is preferentially sensitive to oxidative stress relative to AFG3L2.

We next evaluated whether reduced YME1L results from a stress-induced decrease in YME1L stability. We employed quantitative immunoblotting to measure YME1L stability in SHSY5Y cells treated with cycloheximide (CHX) for 0, 3, or 6 h in the presence or absence of H_2O_2 (Fig 1G and H). In the absence of H_2O_2, YME1L is a stable protein, demonstrating near complete recovery following a 6-h CHX treatment. In contrast, co-administration of CHX and H_2O_2 significantly reduced YME1L protein levels, indicating that H_2O_2 increases YME1L targeting to proteolytic degradation.

YME1L degradation involves the activity of the ATP-independent mitochondrial protease OMA1

We characterized the molecular mechanism of YME1L degradation in isolated mitochondria. YME1L is degraded in isolated mitochondria incubated in the absence of ATP, while AFG3L2 is not affected under these conditions (Fig 2A). The addition of ATP inhibits YME1L degradation in isolated mitochondria (Fig 2A). Identical results were observed in mitochondria purified from mouse livers (Supplementary Fig S2A). Incubating isolated mitochondria with H_2O_2 does not induce YME1L degradation in the presence of ATP, suggesting that ATP depletion as a consequence of oxidative insult, and not the oxidative insult itself, is necessary to induce YME1L degradation (Supplementary Fig S2B). The degradation of YME1L in isolated mitochondria is also inhibited by ADP, but not AMP (Fig 2B). The stabilization of YME1L afforded by ADP cannot be attributed to conversion of ADP to ATP by mitochondrial ATP synthase, as the addition of oligomycin A did not influence ADP-dependent YME1L stabilization (Supplementary Fig S2C). The non-hydrolyzable ATP analog AMP-PNP also inhibits YME1L degradation in isolated mitochondria (Fig 2B). These results suggest that binding of nucleotide stabilizes YME1L against degradation independent of ATP hydrolysis.

YME1L degradation in isolated mitochondria incubated in the absence of ATP is also inhibited by the zinc chelator o-phenanthroline (o-phe) and the divalent cation chelator EDTA (Fig 2C). Inhibitors of serine, cysteine, and aspartic acid proteases did not inhibit YME1L degradation in isolated mitochondria (Supplementary Fig S2D). This suggests that YME1L degradation involves the activity of an ATP-independent zinc metalloprotease. Specifically, a metalloprotease with an active site oriented toward the intermembrane space (IMS), as EDTA cannot cross the inner mitochondrial membrane and only inhibits metalloproteases with IMS-oriented active sites [21]. Currently, the only known ATP-independent zinc metalloprotease embedded in the mitochondrial inner membrane is OMA1. OMA1 is activated by depolarization of the mitochondrial membrane and functions to maintain mitochondrial proteostasis through the degradation of misfolded proteins within the inner membrane [22] and the regulation of mitochondrial dynamics through the stress-induced proteolytic processing of the dynamin-like GTPase OPA1 [23,24].

To interrogate the role of OMA1 in stress-induced YME1L degradation, we measured H_2O_2-dependent YME1L degradation in wild-type ($OMA1^{+/+}$) mouse embryonic fibroblasts (MEFs) and MEFs homozygous for a null allele of $OMA1$ ($OMA1^{-/-}$) [25]. YME1L is efficiently degraded in H_2O_2-treated $OMA1^{+/+}$ MEFs (Fig 2D and E, and Supplementary Fig S2E). The activation of OMA1 in $OMA1^{+/+}$ MEFs was confirmed by the H_2O_2-dependent increase in OPA1 processing from long to short isoforms (arrows in Fig 2D and Supplementary Fig S2E). Alternatively, H_2O_2-dependent YME1L degradation is attenuated, but not completely inhibited, in $OMA1^{-/-}$ MEFs. AFG3L2 degradation was likewise reduced in H_2O_2-treated $OMA1^{-/-}$ MEFs (Fig 2D). *In vitro* YME1L degradation was also attenuated, but not completely inhibited, in mitochondria purified from $OMA1^{-/-}$ MEFs incubated in the absence of ATP (Fig 2F). The activation of OMA1 in these experiments is evident in mitochondria purified from $OMA1^{+/+}$, but not $OMA1^{-/-}$, MEFs by OPA1 processing. This inability to completely inhibit YME1L degradation in $OMA1^{-/-}$ MEFs or $OMA1^{-/-}$ isolated mitochondria suggests that another mitochondrial protease(s) also contributes to stress-induced YME1L degradation. Regardless, our results implicate OMA1 activity in the regulation of YME1L stability during oxidative stress.

Oxidative stress could promote YME1L degradation through alterations in its oligomeric structure. YME1L exists as an ~1 MDa *i*-AAA protease complex in mammals, comparable to the Yme1-Mgr1/3 complex in yeast [8,26,27]. We monitored the YME1L complexes in $OMA1^{+/+}$ and $OMA1^{-/-}$ MEFs treated with or without H_2O_2 using Blue Native polyacrylamide gel electrophoresis (BN-PAGE). YME1L primarily forms an ~1 MDa oligomeric complex in both $OMA1^{+/+}$ and $OMA1^{-/-}$ MEFs, with a small population of smaller YME1L complexes between 720 kDa and 242 kDa (Fig 2G). The addition of H_2O_2 increases the populations of these smaller complexes, indicating that H_2O_2 affects YME1L complex stability (Fig 2G). This suggests that YME1L degradation may proceed through dissociation of the YME1L oligomer, although further mechanistic studies are required to define a specific role for YME1L dissociation in this process.

YME1L stability is sensitive to reduced ATP levels and mitochondrial membrane depolarization

The results shown in Fig 2 suggest that YME1L stability is sensitive to intracellular nucleotide levels and OMA1 activation. In order to separate the contributions of these two parameters on YME1L stability, we treated HEK293T cells with 2-deoxyglucose (2-DG; an inhibitor of glycolysis) and/or carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP; an uncoupler that depolarizes the mitochondrial membrane and activates OMA1). We then measured cellular ATP levels, OMA1 activation (via OPA1 processing), and YME1L protein levels. The addition of 2-DG reduces cellular ATP by 60%, but does not increase OPA1 processing and only modestly decreases YME1L (Fig 3A). The addition of CCCP decreases ATP levels by ~20% and increases OPA1 processing, but again only modestly decreases YME1L. The co-administration of 2-DG and CCCP reduces ATP levels > 90%, increases OPA1 processing, and decreases YME1L by 85%. The addition of 2-DG and/or CCCP does not affect AFG3L2 protein levels in these cells (Fig 3A). These results demonstrate that neither depletion of ATP nor OMA1 activation alone recapitulates the extent of YME1L degradation observed during oxidative stress.
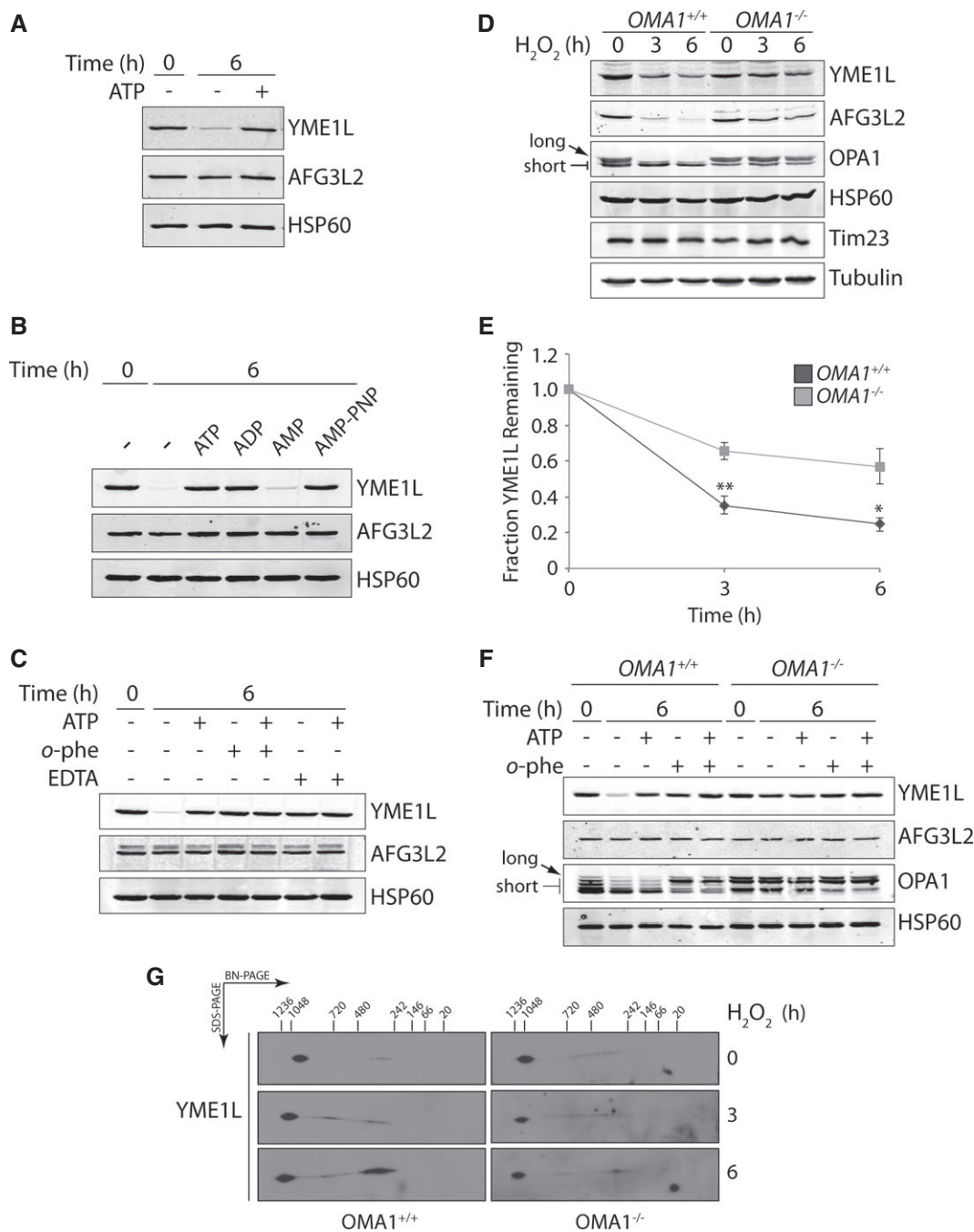
Figure 2. YME1L degradation involves the activity of the ATP-independent mitochondrial protease OMA1.

A   Immunoblot of mitochondria purified from SHSY5Y cells and incubated at 37°C in the presence or absence of ATP (5 mM) for 0 or 6 h, as indicated.

B   Immunoblot of mitochondria purified from SHSY5Y cells and incubated at 37°C for 0 or 6 h in the presence of ATP (5 mM), ADP (5 mM), AMP (5 mM), or AMP-PNP (5 mM), as indicated.

C   Immunoblot of mitochondria purified from SHSY5Y cells and incubated at 37°C in the presence of ATP (5 mM), o-phenanthroline (o-phe; 1 mM), or EDTA (5 mM) for 0 or 6 h, as indicated.

D   Representative immunoblot of lysates prepared from $OMA1^{+/+}$ or $OMA1^{-/-}$ MEFs treated with H_2O_2 (200 μM) for the indicated time. The long and short isoforms of OPA1 are indicated by the arrows.

E   Quantification of normalized YME1L protein levels from immunoblots as shown in (D). Error bars show SEM for $n = 4$. **P-value < 0.01, *P-value < 0.05.

F   Immunoblot of mitochondria purified from $OMA1^{+/+}$ or $OMA1^{-/-}$ MEFs incubated at 37°C in the presence of ATP (5 mM) or o-phenanthroline (o-phe; 1 mM) for 0 or 6 h, as indicated. The long and short isoforms of OPA1 are indicated by the arrows.

G   Immunoblot of BN-PAGE/SDS–PAGE of mitochondria isolated from $OMA1^{+/+}$ and $OMA1^{-/-}$ from cells treated with H_2O_2 (200 μM) for 0, 3, or 6 h, as indicated. These immunoblots were overexposed to observe alterations in YME1L oligomeric structure in these cells despite reductions in YME1L protein levels.

Source data are available online for this figure.

Only a combination of these two treatments induces a robust decrease in YME1L. The inability of OMA1 activation alone to induce YME1L degradation is also shown by the ATP-dependent stabilization of YME1L in isolated mitochondria where OMA1 is active (Fig 2F).

We further explored the relative dependence of YME1L stability on nucleotide depletion and OMA1 activation by treating HEK293T cells cultured in galactose-supplemented media with the ATP synthase inhibitor oligomycin A and/or CCCP. Under these culture conditions, oligomycin A reduces cellular ATP levels, as the majority of ATP is produced through mitochondrial oxidative phosphorylation. Alternatively, CCCP both activates OMA1 and reduces cellular ATP through disruption of the mitochondrial inner membrane potential required for oxidative phosphorylation. The addition of oligomycin A reduces ATP levels > 90%, but does not significantly activate OMA1 or reduce YME1L (Fig 3B). Alternatively, the addition of CCCP alone or in combination with oligomycin A depletes ATP levels > 90%, significantly activates OMA1, and reduces YME1L > 90%. Again, AFG3L2 is not decreased in cells treated with oligomycin A and/or CCCP (Fig 3B).

OMA1 undergoes rapid degradation in response to stresses that depolarize the mitochondrial membrane such as CCCP [22,28]. Thus, we evaluated the relationship between OMA1 and YME1L degradation induced by CCCP in the presence or absence of 2-DG. As observed previously, CCCP induces a reduction in total OMA1 protein levels (Fig 3C) [22,28]. Alternatively, the co-addition of 2-DG with CCCP slows OMA1 degradation and results in the accumulation of a proteolytically processed OMA1 isoform previously shown to retain protease activity (arrows in Fig 3C) [28]. The addition of 2-DG alone does not influence OMA1 stability. These results suggest that CCCP-dependent OMA1 degradation is sensitive to cellular ATP levels. Consistent with this prediction, efficient OMA1 degradation in isolated mitochondria requires the addition of exogenous ATP (Supplementary Fig S3A). Other stresses that induce YME1L degradation such as H_2O_2 and antimycin A in galactose-cultured cells also do not induce OMA1 degradation (Fig 3D and Supplementary Fig S3B), although antimycin A promotes OMA1 cleavage into the active proteolytic fragment. The activation of OMA1 in antimycin-treated cells cultured in galactose is consistent with the depolarization of the mitochondrial membrane in these cells, as shown by reduction in TMRE fluorescence (Supplementary Fig S3C). These results show that OMA1 and YME1L degradation are differentially sensitive to cellular stresses, potentially yielding

distinctive mitochondrial inner membrane proteolytic activities in response to specific pathologic insults.

The results shown in Fig 3 demonstrate that YME1L intracellular stability is sensitive to reductions in cellular ATP and inner membrane depolarization, the latter of which activates OMA1. Combined with the biochemical characterization of YME1L degradation shown in Fig 2, these results suggest a model for the mechanism of stress-induced YME1L degradation whereby a reduction in cellular ATP alters YME1L conformational stability through reduced nucleotide binding, increasing the targeting of YME1L to proteolytic degradation mediated through a process involving active OMA1 and at least one additional mitochondrial protease(s) (Fig 3E).

Loss of YME1L decreases the regulation of mitochondrial proteostasis and sensitizes cells to oxidative insult

Stress-induced YME1L degradation could represent an early step in apoptotic signaling. To test this prediction, we monitored apoptotic signaling by caspase-3 activation and PARP cleavage in cells treated with stresses that induce YME1L degradation. Despite inducing robust YME1L degradation, we observe no caspase-3 activation or PARP cleavage in N2a cells treated with H_2O_2 (Fig 4A) or HEK293T cells cultured in galactose-supplemented media and treated with antimycin A (Supplementary Fig S3B). Conversely, staurosporine robustly activates apoptosis without significantly affecting YME1L stability (Fig 4A). Alternatively, YME1L degradation, caspase-3 activation, and PARP cleavage are all observed in As(III)-treated SHSY5Y cells (Supplementary Fig S4A). These results indicate that stress-induced YME1L degradation does not correspond with the initiation of apoptosis. Furthermore, YME1L protein levels recover over a 24-h period following a H_2O_2 insult, mirroring the recovery of full-length OPA1 (Fig 4B) [29]. This suggests that YME1L degradation does not represent a 'point of no return' for cell death.

Alternatively, YME1L degradation could decrease the capacity for cells to regulate inner membrane proteostasis. To explore this potential consequence of YME1L degradation, we monitored the impact of oxidative stress on YME1L-mediated regulation of the TIM23 mitochondrial protein import complex [30]. Mammalian TIM23 forms two exclusive complexes containing distinct core interactions between the subunit Tim23 and one of the two mammalian paralogs of yeast Tim17, Tim17A, or Tim17B [31]. Previous work showed that Tim17A is a stress-regulated TIM23 subunit that is rapidly degraded by YME1L in response to eIF2α phosphorylation-dependent
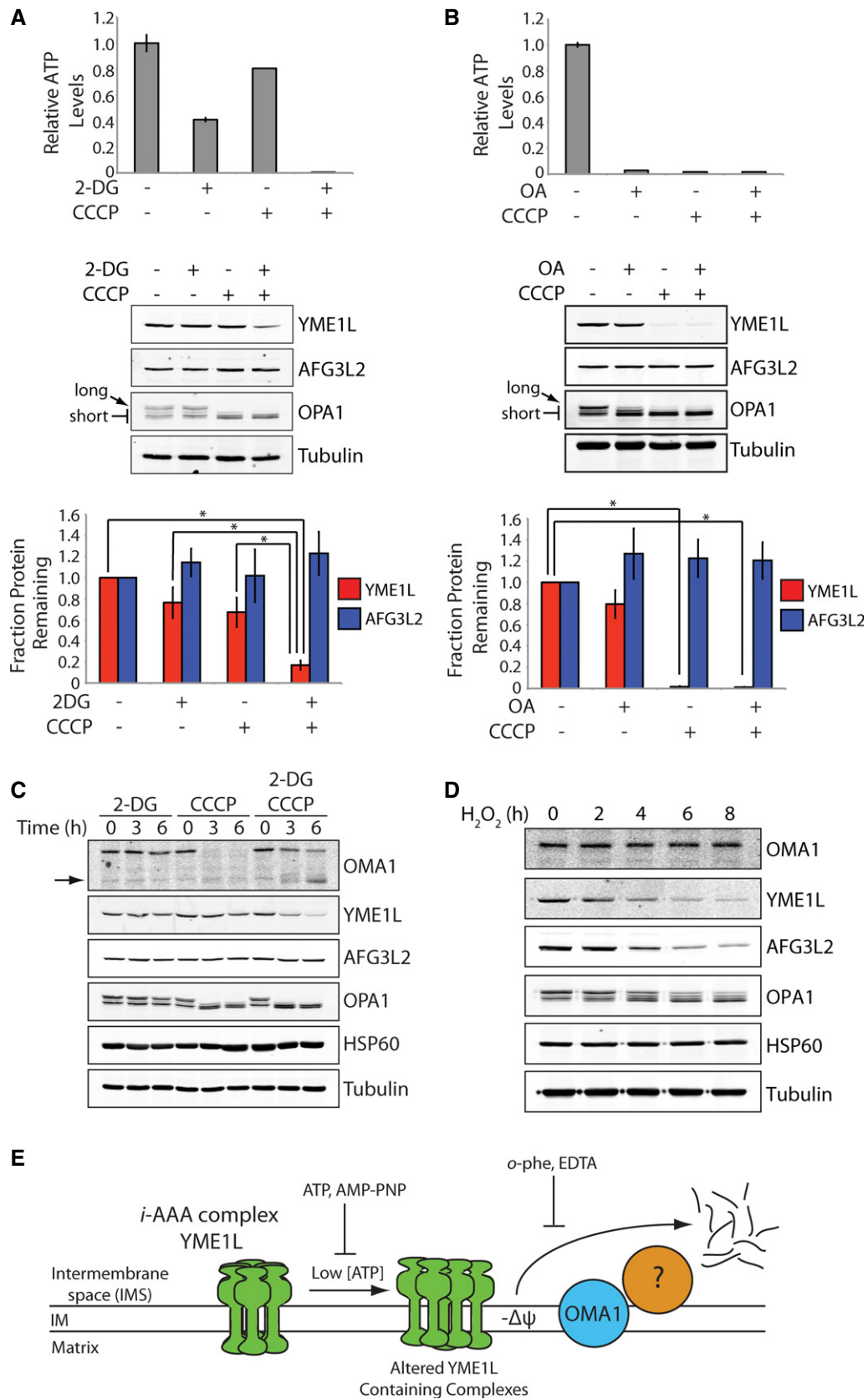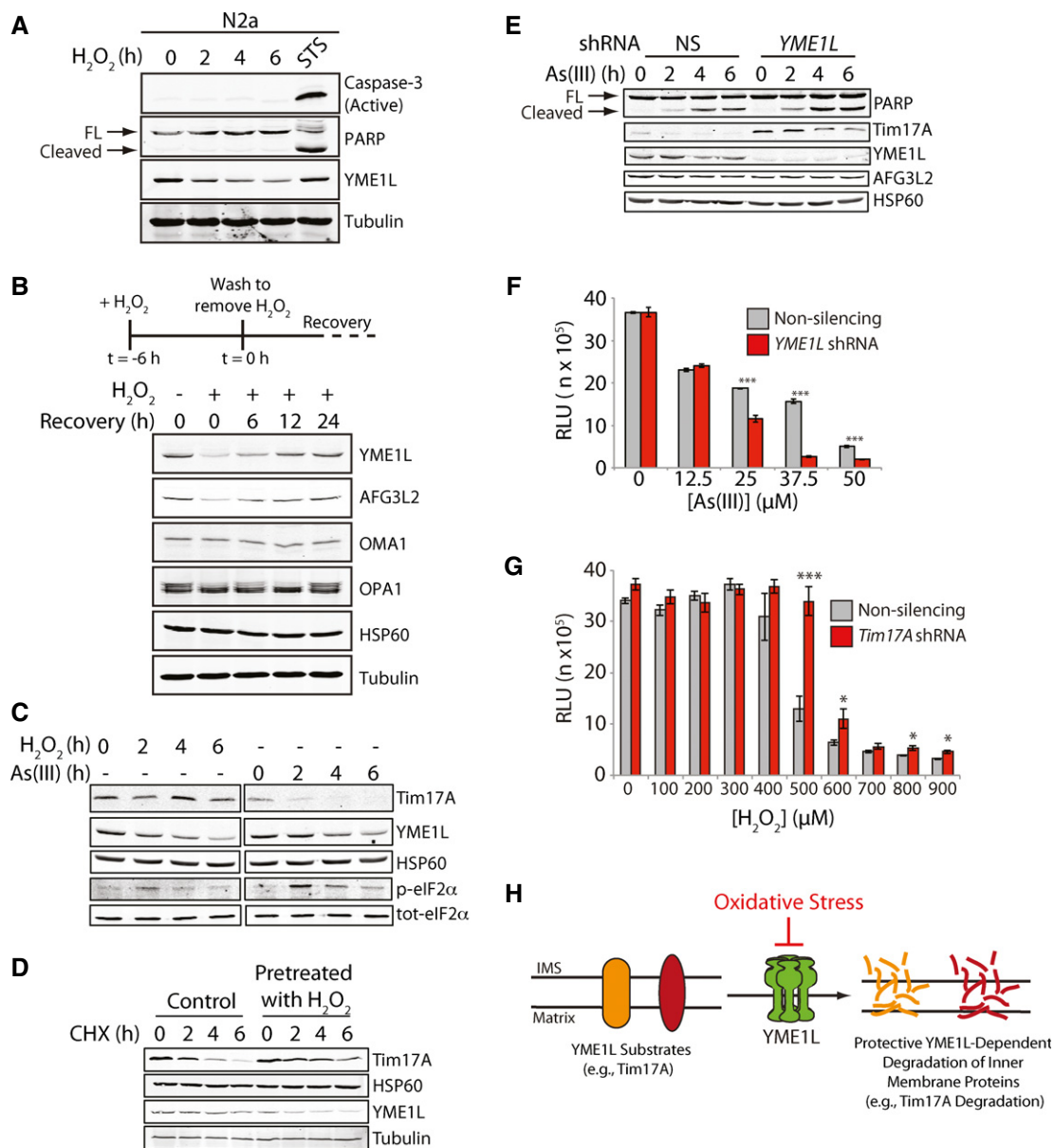
Figure 3.

Figure 4. Loss of YME1L decreases cellular capacity to regulate mitochondrial proteostasis and sensitizes cells to oxidative insult.

A Immunoblot of lysates prepared from N2a cells treated with H_2O_2 (200 μM) for the indicated times. Lysates prepared from N2a cells treated with staurosporine (STS; 1 μM, 6 h) are shown as a control for apoptosis. Full-length (FL) and cleaved PARP are shown by the arrows.

B Immunoblot of lysates from SHSY5Y cells pretreated with H_2O_2 (200 μM) for 6 h, as indicated, then washed, and incubated in fresh media for the indicated times. An illustration of the experimental design for this experiment is shown above.

C Immunoblot of lysates prepared from HEK293T cells treated with the H_2O_2 (100 μM) or As(III) (50 μM) for the indicated time. Note the H_2O_2-dependent increase in eIF2α phosphorylation, but lack of Tim17A degradation in these cells.

D Immunoblot of lysates prepared from HEK293T cells treated with cycloheximide (CHX; 50 μg ml^{-1}) in the presence or absence of an H_2O_2 pretreatment (100 μM; 3 h).

E Immunoblot of lysates prepared from SHSY5Y cells stably expressing non-silencing (NS) shRNA or *YME1L* shRNA. Cells were treated with As(III) (50 μM) for the indicated time. Full-length (FL) and cleaved PARP are shown by the arrows.

F Graph showing the viability of SHSY5Y cells expressing non-silencing (NS) shRNA or *YME1L* shRNA treated with increasing concentrations of As(III) for 24 h. Cell viability was measured using CellTiter-Glo. Error bars show SEM for $n = 3$. ***P-value < 0.001.

G Graph showing the viability of HEK293T cells expressing non-silencing (NS) shRNA or *TIM17A* shRNA treated with increasing concentrations of H_2O_2 for 24 h. Cell viability was measured using CellTiter-Glo. Error bars show SEM for $n = 3$. ***P-value < 0.001, *P-value < 0.05. These results are representative of 3 independent experiments.

H Illustration showing that oxidative insults that induce YME1L degradation decrease protective YME1L-dependent, stress-regulated degradation of inner mitochondrial membrane proteins. This decreases cellular capacity to maintain critical mitochondrial inner membrane functions during stress and promotes cellular death.

Source data are available online for this figure.

translational attenuation [30]. However, Tim17B is not subject to this regulation. Thus, YME1L-mediated degradation of Tim17A reduces the population of active TIM23 complexes containing a core Tim23–Tim17A interaction without impacting TIM23 complexes containing a core Tim23–Tim17B interaction [30]. This provides a mechanism for cells to sensitively attenuate, but not completely inhibit, TIM23-dependent protein import in response to pathologic insult [30]. The attenuation in mitochondrial protein import afforded by Tim17A degradation is predicted to promote mitochondrial proteostasis through mechanisms such as reducing the population of newly synthesized unfolded proteins entering mitochondrial during stress and by promoting transcriptional remodeling of mitochondrial proteostasis pathways [30,32]. Consistent with a protective role for Tim17A degradation, reducing Tim17A levels increases cellular viability in response to mitochondrial insults such as paraquat [30]. The benefits attributed to YME1L-dependent Tim17A degradation are in contrast to those observed for the degradation of Tim23, the core channel forming subunit of TIM23 complexes, which is observed during cell death in the presence of caspase inhibitors [33]. Since Tim23 is an essential subunit for *all* TIM23 complexes, reductions in Tim23 will decrease the cellular populations of *all* active TIM23 complexes, completely inhibiting TIM23-dependent mitochondrial protein import [33]. Consistent with the differential impacts of reducing Tim17A or Tim23 on mitochondrial protein import, RNAi depletion of *TIM23* inhibits cellular proliferation, while *TIM17A* depletion does not impact cellular growth [30,33]. These results serve to further highlight the protective role for selective, YME1L-dependent Tim17A degradation in regulating TIM23-dependent mitochondrial protein import, promoting mitochondrial proteostasis and increasing cellular viability in response to stress [30].

H_2O_2 is known to induce stress-regulated eIF2α phosphorylation [34] and thus should induce Tim17A degradation. Interestingly, despite inducing eIF2α phosphorylation, Tim17A is not degraded in H_2O_2-treated SHSY5Y cells where YME1L is rapidly degraded (Fig 4C). This is in contrast to the degradation of Tim17A observed in As(III)-treated cells. The capacity for As(III) to induce Tim17A degradation despite also promoting YME1L degradation likely reflects the slower rate of As(III)-induced YME1L degradation relative to H_2O_2 (c.f. Fig 1D and G). The inability for H_2O_2 to induce Tim17A degradation could be attributed to lower levels of eIF2α phosphorylation induced by this treatment, as compared to As(III). In order to better define the capacity for H_2O_2 to inhibit YME1L-dependent Tim17A degradation, we measured Tim17A levels in SHSY5Y cells pretreated with H_2O_2 and subjected to CHX-dependent translational attenuation—a treatment that induces Tim17A degradation independent of eIF2α phosphorylation [30]. Pretreatment with H_2O_2 attenuates CHX-dependent Tim17A degradation, consistent with H_2O_2-induced YME1L degradation inhibiting Tim17A regulation (Fig 4D). H_2O_2-dependent YME1L degradation is not affected by *TIM17A* depletion, showing that YME1L degradation occurs upstream of Tim17A (Supplementary Fig S4B). Importantly, the addition of H_2O_2 to isolated mitochondria does not inhibit the YME1L-mediated, ATP-dependent degradation of Tim17A (Supplementary Fig S4C). This shows that H_2O_2 does not antagonize the ability for YME1L to degrade Tim17A, but instead reflects the predicted reduction in YME1L activity in cells treated with H_2O_2. Collectively, these results indicate that H_2O_2 inhibits YME1L

proteolytic activity, compromising the capacity for cells to regulate mitochondrial protein import and global mitochondrial inner membrane proteostasis during oxidative stress.

The decreased capacity for YME1L to regulate mitochondrial inner membrane proteostasis should sensitize cells to stress. As reported previously [8], *YME1L* depletion sensitized SHSY5Y cells to apoptosis induced by staurosporine (STS) shown by increased PARP cleavage (Supplementary Fig S4D). Likewise, PARP cleavage was enhanced in *YME1L*-depleted cells following As(III) treatment (Fig 4E). *YME1L* depletion also reduced cell viability in response to As(III) (Fig 4F). This suggests that the loss of YME1L-dependent regulation of mitochondrial inner membrane proteostasis through activities such as Tim17A degradation sensitizes cells to oxidative insult. Consistent with this prediction, depletion of *TIM17*A in HEK293T cells—mimicking stress-regulated Tim17A degradation—increases cellular viability in response to H_2O_2, suggesting that inhibition of YME1L-dependent Tim17A degradation reduces cellular capacity to regulate mitochondria in response to oxidative insult (Fig 4G). Collectively, the results shown in Fig 4C–G indicate that loss of YME1L activity reduces cellular capacity to regulate mitochondrial inner membrane proteostasis during stress and increases cellular sensitivity to pathologic insult.

Conclusions

YME1L is a critical regulator of essential mitochondrial functions [8,9,11]. Here, we show that YME1L is rapidly degraded through a mechanism involving the mitochondrial protease OMA1 in response to oxidative insults that deplete cellular ATP and depolarize the mitochondrial inner membrane. Reducing YME1L activity compromises the stress-dependent degradation of YME1L substrates such as Tim17A, impairing the capacity to regulate inner mitochondrial membrane proteostasis and function during stress (Fig 4G). The deficiencies in mitochondrial proteostasis and quality control caused by loss of YME1L increase cellular sensitivity to subsequent oxidative stress, as indicated by the increased stress sensitivity of *YME1L*-depleted cells. While our results show that oxidative stress-induced YME1L degradation is a mechanism that can contribute to the pathologic mitochondrial dysfunction involved in human diseases, YME1L degradation could also play a regulatory role in adapting mitochondrial function during conditions of stress. For example, it was recently shown that YME1L activity is needed for the *de novo* cleavage of OPA1 to mediate fusion events [10]. Therefore, YME1L degradation may provide a mechanism to sequester terminally damaged mitochondria away from the healthy pool thereby restricting them to clearance via mitophagy, although additional studies will be required to define these potentially protective consequences of stress-induced YME1L degradation.

Oxidative stress and dysregulation of mitochondrial inner membrane proteins are intricately associated in the onset and pathology of human diseases including cancer, diabetes, and neurodegenerative disorders [35–38]. Despite this association, the relationship between mitochondrial dysfunction and oxidative insults remains poorly defined. Our identification of YME1L as an oxidative stress-sensitive mitochondrial protease reveals a mechanism that can contribute to pathologic dysregulation of mitochondrial inner membrane functions involved in the pathogenesis of these diseases.

Materials and Methods

Cell lines and culture conditions

SHSY5Y cells were grown in Dulbecco's modified Eagle's medium/ Ham's F-12 Nutrient Mixture (DMEM/F12) supplemented with 4 mM L-glutamine, 10% FBS, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37°C and 5% CO$_2$. HEK293T, N2a, HeLa, and mouse embryonic fibroblasts (MEFs) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine, 10% FBS, 100 U ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin at 37°C and 5% CO$_2$. For cells grown in galactose and 2-deoxy-D-glucose treatments, see Supplementary Methods. Cell lines with stable shRNA knockdown of *YME1L* and *Tim17A* were previously described [30].

Lysate preparation and immunoblot analysis

Lysate preparation and immunoblotting were performed as described previously [30]. Primary antibodies were obtained commercially and used as described in Supplementary Methods.

Mitochondrial isolation and assays

Mitochondrial were purified as described previously [39]. Isolated mitochondria were handled as described previously and treated as indicated [30].

Blue Native PAGE

Blue Native PAGE was performed as described previously [30,40]. Membranes were visualized using HRP-conjugated secondary antibody (Cell Signaling) and detected by enhanced chemiluminescence with Luminata Forte (Millipore) using film.

Quantification of relative ATP levels and determination of cell viability

Cells were plated in poly-D-lysine-coated 12-well plates and treated as indicated for ATP depletion or OMA1 activation. Relative ATP levels were quantified using CellTiter-Glo (Promega). Briefly, treated cells were washed into DPBS and lysed by the addition of CellTiter-Glo reagent. Lysate was transferred to a flat black 96-well plate and incubated in the dark for 10 min to stabilize signal. For cell viability assays, cells were directly seeded into flat black 96-well plates and treated as indicated before lysis with CellTiter-Glo reagent and handled similarly. Luminescence intensity was read in an Infinite F200 PRO plate reader (Tecan) and background corrected. All measurements were made in biologic triplicate.

Supplementary information for this article is available online: http://embor.embopress.org

Author contributions

RLW guided the project. TKR, JMS, and RLW designed and carried out the experiments, and analyzed the data. TKR and RLW prepared the figures, discussed the data, and wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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