Kennedy disease, a degenerative disorder caused by an expanded glutamine tract, is mediated by misfolding of the mutant androgen receptor (AR) protein, a process that may disrupt proteasome function. We hypothesized that this might lead to endoplasmic reticulum (ER) stress and induction of the unfolded protein response (UPR), a complex physiologic pathway that regulates cell survival. To test this hypothesis, we used amino-terminal fragments of wild type (AR160Q) or mutant (AR112Q) AR that triggered glutamine length-dependent cell death and activated an ER stress-inducible promoter. To evaluate the role of the UPR, we examined the contributions of three proximal sensors of ER stress: activating transcription factor 6 (ATF6), inositol requiring 1 (IRE1), and PKR-like endoplasmic reticulum kinase (PERK). AR112Q toxicity was significantly increased by a dominant negative ATF6 mutant and significantly decreased by a constitutively active ATF6 mutant, indicating that ATF6 promoted cell survival. In contrast, co-transfection with three separate IRE1α dominant negative mutants failed to alter glutamine length-dependent toxicity, suggesting that this arm of the UPR did not significantly affect AR112Q induced cell death. Activation of PERK, an ER transmembrane protein that functions as the third proximal UPR sensor, promoted glutamine length-dependent toxicity. Although nuclear localization sequence- and nuclear export sequence-targeted proteins both activated the UPR, this pathway more potently influenced toxicity when proteins were targeted to the cytoplasm. Taken together, our data demonstrate that the UPR is activated in cells expressing long glutamine tracts and that this pathway modulates polyglutamine toxicity.

A diverse group of neurodegenerative diseases is characterized by the presence of aggregated or misfolded proteins that accumulate either within neurons or in the neuropil. Among these disorders are ones caused by expansions of CAG/glutamine tracts (1). Kennedy disease, a member of this group, is a chronic, progressive neuromuscular disease characterized by proximal muscle weakness, atrophy, and fasciculations (2). The cause of this disorder is the expansion of a CAG repeat in the first exon of the androgen receptor (AR)1 gene (3). The mutant AR protein misfolds and aggregates, leading to both a toxic gain-of-function and a partial loss of normal function (2, 4). Studies in cell-based and animal models have suggested that the misfolded, mutant AR, like other proteins with expanded glutamine tracts, may exert toxic affects by disrupting the ubiquitin-proteasome pathway, by sequestering other factors such as transcriptional co-regulators or by impairing synaptic function and axonal transport (5–7).

Inhibition of the ubiquitin-proteasome pathway may contribute to neuronal dysfunction and death by disrupting signaling pathways and by limiting the capacity of cells to degrade misfolded or damaged proteins. Proteasome inhibition by the expanded glutamine androgen receptor occurs in a ligand-dependent manner in cell culture (8) and may contribute to the testosterone-dependent appearance of a phenotype in transgenic mouse models of Kennedy disease (9–11). Truncated protein fragments containing long glutamine tracts also inhibit proteasome function (12, 13) and form aggregates that colocalize with molecular chaperones and proteasome components. Exogenously expressed Hsp70 and Hsp40 enhance degradation of these mutant proteins in cell culture (14), and overexpressed Hsp70 decreases disease severity in transgenic mouse models of Kennedy disease (15) and SCA1 (16), another polyglutamine expansion disorder.

Among the targets for degradation by the proteasome are those generated in the endoplasmic reticulum (ER) during the processing of soluble and membrane-bound proteins (17). These misfolded products are retrotranspoated to the cytosol for degradation by the proteasome through a process known as ER-associated degradation. That ER-derived proteins may contribute to neuronal loss has been suggested by studies in a Drosophila model of polyglutamine disease (18). The accumulation of misfolded proteins in the ER through a variety of mechanisms including proteasome inhibition can activate the unfolded protein response (UPR), a complex network of physiologic responses that may either relieve ER stress or activate apoptosis (19–21).

UPR activation is mediated by three ER transmembrane proteins that function as proximal sensors, designated activating transcription factor 6 (ATF6), IRE1, and PKR-like endo-

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1 The abbreviations used are: AR, androgen receptor; ER, endoplasmic reticulum; UPR, unfolded protein response; ATF6, activating transcription factor 6; IRE1, inositol requiring 1; PERK, PKR-like endoplasmic reticulum kinase; eIF2α, eukaryotic translation initiation factor 2α; BIP, binding immunoglobulin protein; FMKR, fluoromethyl ketone; Z, benzylxycarbonyl; UPR, unfolded protein response element; DN, dominant negative; CA, constitutively active; NES, nuclear export sequence; NLS, nuclear localization sequence; JNK, c-Jun NH2-terminal kinase.
plasmic reticulum kinase (PERK) (22). Two mammalian orthologs of yeast inositol requiring 1 have been characterized and are designated IRE1α and IRE1β (23, 24). Although most cell types express IRE1α, epithelial cells of the gastrointestinal tract are the primary sites of IRE1β expression. In the presence of ER stress, IRE1 dimerizes, autophosphorylates, and activates intrinsic RNase activity (25). Activated IRE1 in turn splices X-box-binding protein 1 mRNA to generate a potent transcriptional activator. Like IRE1, ATF6 is an ER transmembrane protein that functions as a proximal sensor for the UPR. In the presence of ER stress, ATF6 transits to the Golgi, where it is cleaved to generate a cytosolic fragment that migrates to the nucleus and functions as a transcriptional activator (26). Both of these pathways lead to activation of DNA-binding proteins that recognize promoter elements in ER stress-activated genes (27–30). Like IRE1 and ATF6, PERK is an ER membrane protein that functions as a proximal sensor for the UPR. It is cleaved to generate a cytosolic fragment that migrates to the nucleus and functions as a transcriptional activator (26).

Prior studies have suggested that expression of proteins with expanded glutamine tracts results in ER stress as marked by cleavage of caspase 12 (32) and phosphorylation of IRE1 (33). Although these observations suggest that proteins with expanded glutamine tracts activate the UPR, the consequences of this activation are not well characterized. Here we have used amino-terminal fragments of the androgen receptor containing either normal or expanded glutamine tracts to explore the role of the UPR as a modulator of glutamine length-dependent toxicity. We demonstrate glutamine length-dependent activation of the UPR as detected by induction of an ER stress-responsive promoter and by phosphorylation of endogenous PERK. We further demonstrate that ATF6, IRE1, and PERK, three proximal sensors of ER stress, have varied effects on the survival of cells expressing the mutant AR. These data identify the UPR as an important physiologic pathway that modulates glutamine length-dependent toxicity, and suggest that this pathway may offer therapeutic targets for the treatment of the polyglutamine diseases.

**EXPERIMENTAL PROCEDURES**

**Materials—**HeLa cells were from American Type Culture Collection (Manassas, VA). Plasmids encoding ARQ16 and AR112Q were a gift from Diane Merry (Thomas Jefferson University). Nuclear localization sequence (NLS)-targeted AR constructs were a gift from Kenneth Fishbeck (National Institutes of Health). IRE1α mutants, UPRE luciferase, and BiP constructs were a gift of Randal Kaufman (Univ. of Michigan). PERK mutant was a gift of Shiyong Yu (Ohio University). ATF6 mutants were a gift from Claudius Vincenz (University of Michigan). Antibodies against AR (N-2O), GRP 78 (N-20), and BiP constructs were a gift of Randal Kaufman (Univ. of Michigan).

**Antibodies**—Antibodies against AR (N-2O), GRP 78 (N-20), and BiP constructs were a gift of Randal Kaufman (Univ. of Michigan). Antibodies against AR (N-2O), GRP 78 (N-20), and β-tubulin were from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibody HA.11 was from Covance Research Products (Princeton, NJ). Antibodies against phosphorylated eIF2α were from Cell Signaling (Beverly, MA). Antibody against phosphorylated eIF2α was from BIOSOURCE (Camarillo, CA). The antibody against ATF6 was from Imgenex (San Diego, CA). Fluorescein isothiocyanate and Texas red-conjugated secondary antibodies were from Jackson ImmunoResearch (West Grove, PA). TRIZol and reverse transcriptase were from Invitrogen. Amino allyl-UTP was from Sigma, and Cy3 and Cy5 NHS ester dyes were from Amersham Biosciences. 5-VAD-FMK was from Calbiochem.

**Nuclear Export Sequence (NES)-targeted AR Constructs—**A nuclear export sequence encoding amino acids LALKLAGLDI (34) was added in-frame to the 5′-end of non-targeted AR16Q and AR112Q. This sequence was preceded by an initiation methionine and was added into EcoRI and EagI sites of the original vectors after annealing oligonucleotides 5′-AATTTCCACCATGGTTAGCTGGCTTTAGTTACGAGGATTAGACATCCTCC and 5′-GGGCCCAGGATTCTCTCCCTGAATTCAGTCTGATGCTG. This cloning strategy resulted in the substitution of the first 11 amino acids of AR with the NES sequence.

**Cell Transfection—**HeLa cells grown in minimum essential medium α supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin-glutamine were transfected using 5 μl of FuGENE 6 (Roche Applied Science) and 1 μg of DNA/well of a 6-well dish according to the manufacturer’s instructions.

**Western Blot Analysis—**Cells were harvested, washed with phosphate-buffered saline, and lysed in radioimmuno precipitation assay buffer. Protein samples were electrophoresed through a 10% SDS-polyacrylamide gel and transferred to Immobilon-P membranes using a semi-dry transfer apparatus. Immunoreactive proteins were detected by chemiluminescence.

**Fig. 1. Glutamine length-dependent cell death and UPR activation.** A, AR112Q activates caspase 3. HeLa cells were transfected with expression constructs encoding AR16Q (black bars) or AR112Q (white bars), or with control vector (gray bars). Caspase 3 activity was determined at the indicated times posttransfection by measuring cleavage of the fluorescent substrate DEVD-AFC. Data are mean ± S.D. B, the percentage of dead cells was determined 48 h posttransfection by staining with the live/dead assay. C, glutamine length-dependent induction of UPRE-luciferase. HeLa cells were co-transfected with AR16Q (black bars) or AR112Q (white bars) plus UPRE-luciferase (38). Transfection efficiency was normalized to SV40- Renilla luciferase activity. At the indicated times posttransfection, cells were harvested, and luciferase activity was determined. Data are mean ± S.D.
Immunofluorescence Microscopy—Cells cultured on chambered slides were fixed with methanol at −20 °C and stained. Images were captured using a Zeiss LSM 510 confocal microscope under a 63× water immersion objective or using a Zeiss Axiosplan 2 imaging system under a 40× objective. For quantification, triplicate experiments were scored in a blind manner, with >100 cells examined per treatment group in each experiment.

Luciferase Assay—HeLa cells were transfected by calcium phosphate using Promega-mammalian transfection system (Promega, Madison, WI) according to the manufacturer’s instructions. Briefly, cells were seeded in 6-well plates and co-transfected with vectors encoding Renilla (10 ng) and firefly luciferase (0.5 μg) plus the indicated plasmids, using a Zeiss Axiosplan 2 imaging system under a 63× immersion objective or using a Zeiss Axiosplan 2 imaging system under a 40× objective. For quantification, triplicate experiments were scored in a blind manner, with >100 cells examined per treatment group in each experiment.

Cell Viability and Caspase Assay—Cell viability was assessed 48 h posttransfection using the Live/Dead Viability/Cytotoxicity Kit (Molecular Probes, Eugene, OR). Following staining, >100 cells were scored using a Zeiss Axiosplan 2 imaging system. Caspase 3 activity was determined at the indicated times posttransfection by measuring cleavage of the fluorescent substrate DEVD-AFC using the ApoTarget caspase 3/CPP 32 fluorometric protease assay kit (BIOSOURCE) according to the manufacturer’s instructions. Fluorescence intensity was measured using a Fluoroskan Ascent FL fluorometer (Thermo Electron Corp.). Primary data analysis was done using the GenePix software package (Axon Instruments, Foster City, CA).

RESULTS

The UPR Is Activated by AR112Q and Effects Toxicity—We first confirmed that exogenously expressed amino-terminal AR fragments trigger cell death in a glutamine length-dependent manner. These truncated AR constructs encode ~50 amino acids flanking glutamine tracts of either 16 or 112 residues, and include a carboxyl-terminal hemagglutinin tag (AR16Q or AR112Q, respectively) (37). When transiently expressed in HeLa cells, these proteins induced glutamine length-dependent cell death as detected by activation of caspase 3 that peaked 48 h posttransfection (Fig. 1A) and by directly counting dead cells as identified by staining with the live/dead assay (Fig. 1B).

We used this cell culture model to test the hypothesis that the UPR is activated by proteins with expanded glutamine...
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AFT6 nuclear staining (Fig. 2A). These data indicated that signaling through ATF6 primarily decreased AR112Q-mediated caspase 3 activation (Fig. 2B). To determine whether IRE1 impacts glutamine length-dependent activation of caspase 3, HeLa cells were co-transfected with AR16Q, AR112Q, or empty vector plus the indicated IRE1DN mutants. Caspase 3 activity was determined 48 h posttransfection. Data are mean ± S.D. Co-transfection with IRE1α DN mutants did not significantly alter caspase 3 activation by AR112Q (p > 0.05 by unpaired t test).

FIG. 3. Caspase activation by AR112Q is not modulated by IRE1. A, IRE1α DN mutants inhibit induction of UPRE-luciferase by tunicamycin (Tm). HeLa cells were co-transfected with UPRE-luciferase and one of three IRE1α DN mutants encoding proteins with a carboxyl-terminal truncation (delta C) or with lysine to alanine substitutions (K509A, K907A). Transfection efficiency was normalized to SV40-Benilla luciferase activity. 24 h posttransfection, cells were treated with 10 μg/ml Tm or vehicle control for 18 h and then harvested for determination of luciferase activity. Data are mean ± S.D. B, IRE1α DN mutants do not alter glutamine length-dependent activation of caspase 3. HeLa cells were co-transfected with AR16Q, AR112Q, or empty vector plus the indicated IRE1α mutants. Caspase 3 activity was determined 48 h posttransfection. Data are mean ± S.D. Co-transfection with IRE1α DN mutants did not significantly alter caspase 3 activation by AR112Q (p > 0.05 by unpaired t test).

tracts and modulates their toxicity. We initially sought to determine whether amino-terminal AR fragments activated an ER stress-responsive promoter element in a glutamine length-dependent manner. To accomplish this, we used a UPR element (UPRE)-luciferase construct (38). Although this element was initially characterized as an ATF6 binding site, it was subsequently shown to bind X-box-binding protein 1 (28). HeLa cells were co-transfected with AR16Q or AR112Q plus a UPRE-luciferase reporter construct containing five multimerized consensus binding sites (38). We observed time and glutamine length-dependent luciferase activation (Fig. 1C). Luciferase activity was first detected 72 h posttransfection and continued to increase at 96 h posttransfection, suggesting that the UPR was induced in glutamine length-dependent manner in transfected cells.

We next determined the specific effects of proximal UPR sensors on glutamine length-dependent toxicity. To assess ATF6 activation, NIH 3T3 cells transfected with AR16Q or AR112Q were co-stained with antibodies specific for AR and ATF6. Immunofluorescence and quantification revealed that fewer than 5% of cells expressing AR16Q had nuclear ATF6 staining whereas ~20% of AR112Q-expressing cells exhibited ATF6 nuclear staining (Fig. 2A). Similar results were obtained using HEK 293 cells (not shown). These data indicated that ATF6 was activated in a glutamine length-dependent manner. To determine the influence of ATF6 on AR112Q toxicity, we used previously characterized dominant negative (DN) and constitutively active (CA) ATF6 mutants (39). Expression of AR112Q plus AFT6 DN significantly increased toxicity compared with expression of AR112Q alone as measured by caspase 3 activation (Fig. 2B) and by live/dead assay (Fig. 2C). In contrast, expression of ATF6 CA significantly decreased AR112Q-mediated caspase 3 activation (Fig. 2D). These data indicated that signaling through ATF6 primarily protected cells from glutamine length-dependent toxicity.

The transcriptional activator generated by ATF6 cleavage is known to modulate expression of several genes including the ER chaperone BiP, or binding Ig protein. BiP plays an important regulatory role for the UPR by binding to the luminal domains of IRE1, ATF6, and PERK, thereby preventing their activation (22). As unfolded proteins accumulate in the ER lumen, BiP may be sequestered away from these proximal sensors to allow for UPR induction. Because the ATF6 CA mutant up-regulated the expression of endogenous BiP (Fig. 2E), we sought to determine whether increased expression of BiP was sufficient to mediate the protective effects of ATF6 on AR112Q toxicity. Although overexpression of BiP significantly decreased the low level of caspase 3 activation by AR16Q, it did not significantly alter caspase 3 activation by AR112Q (Fig. 2F). Similar results were obtained following transfection with a wide range of plasmid concentrations (data not shown). These data suggested that BiP up-regulation was not sufficient to mediate the protective effects of ATF6 and that other factors were also required to promote the survival of cells expressing AR112Q. The observed glutamine length-dependent induction of UPRE-luciferase (Fig. 1C) indicated that IRE1 is also activated in cells expressing AR112Q. Therefore, we next evaluated whether IRE1 impacts glutamine length-dependent activation of caspase 3. To accomplish this we used three previously characterized IRE1α DN mutants (38). The K509A and K907A point mutants abolish IRE1α kinase and RNase activities, respectively, whereas the δC mutant contains a carboxyl-terminal truncation and lacks both of these functional domains. All three mutants acted as expected in our system and inhibited UPRE activation by tunicamycin, a known inducer of ER stress (Fig. 3A). To determine whether IRE1 was necessary for glutamine length-dependent activation of caspase 3, HeLa cells...
FIG. 4. PERK promotes AR112Q toxicity. A, PERK is phosphorylated in cells expressing AR112Q. HeLa cells expressing AR16Q (upper panel) or AR112Q (lower panel) were identified using the AR antibody N20 (Texas red, left column). Phosphorylation of endogenous PERK was detected by a phosphoPERK specific antibody (fluorescein isothiocyanate, middle column). Merged images are shown in right column. B, quantification of AR16Q or AR112Q positive HeLa cells expressing phosphoPERK. Data are mean ± S.D. C and D, PERK DN mutant inhibits AR112Q toxicity. HeLa cells were co-transfected with AR16Q or AR112Q plus a PERK DN mutant that lacks the carboxyl-terminal luminal domain, or vector control. C, caspase 3 activity was measured 48 h posttransfection. Co-expression of AR112Q and PERK DN significantly decreased caspase 3 activity compared with expression of AR112Q alone (p < 0.03 by unpaired t test). Data are mean ± S.D. D, the percentage of dead cells was determined 48 h posttransfection by staining with the live/dead assay. Co-expression of AR112Q and PERK DN significantly decreased the percentage of dead cells compared with expression of AR112Q alone (p < 0.001 by unpaired t test). Data are mean ± S.D. E, PERK DN mutant does not alter expression of AR constructs. HeLa cells were co-transfected with AR16Q (lanes 1 and 3) or AR112Q (lanes 2 and 4), plus a PERK DN mutant (lanes 3 and 4) or vector control (lanes 1 and 2). Lysates were collected 48 h posttransfection and evaluated by Western blot for expression of AR (top panel) and β-tubulin (bottom panel).
were co-transfected with AR16Q or AR112Q plus one of three IRE1α DN mutants or empty vector (Fig. 3B). No significant change in caspase 3 activity was detected in cells co-transfected with these IRE1α mutants. Similarly, no statistically significant change in AR112Q toxicity was detected by directly counting dead cells identified by the live/dead assay (data not shown). Taken together, these data demonstrated that IRE1 did not significantly impact AR112Q toxicity.

We next sought to evaluate the activation of PERK, the third proximal UPR sensor. To assess PERK activation, HeLa cells transfected with AR16Q or AR112Q were co-stained with AR and phosphoPERK-specific antibodies. Immunofluorescence and quantification revealed that fewer than 7% of AR16Q-positive cells expressed phosphoPERK, whereas ~25% of AR112Q-positive cells expressed phosphoPERK (Fig. 4, A and B). Co-expression of a PERK DN mutant (40) significantly decreased AR112Q toxicity as measured by caspase 3 activation and by live/dead assay (Fig. 4, C and D), indicating that this arm of the UPR triggered a proapoptotic response. This effect of the PERK DN mutant was not mediated by altering expression of the truncated AR proteins (Fig. 4E), suggesting that other downstream effectors induced by PERK promoted cell death.

The above experiments indicated that modulating activity of the proximal UPR sensors ATF6 and PERK influenced the toxicity of AR112Q. As a complementary approach, we determined whether a block of AR112Q toxicity would accentuate UPR activation. Cells expressing AR16Q or AR112Q were treated with the pan-caspase inhibitor Z-VAD-FMK at a concentration that prevented glutamine length-dependent toxicity as measured by caspase 3 activation (supplemental Fig. 1). Treatment of AR112Q-expressing cells with Z-VAD-FMK led to the marked accumulation of phosphorylated eIF2α and the modest accumulation of BiP (Fig. 5), markers of UPR activation.

**Intracellular Localization and UPR Activation**—We were next interested in evaluating whether intracellular localization of the expanded glutamine tract effects UPR activation or the ability of the UPR to modulate toxicity. To accomplish this we used previously characterized constructs containing the same truncated AR fragments with the addition of a NLS (41). In addition, we generated a similar set of constructs containing a NES at the amino terminus. The presence of these targeting sequences resulted in the localization of the encoded proteins to the expected subcellular compartments (Fig. 6A). Both the NES- and NLS-targeted constructs triggered glutamine length-dependent caspase 3 activation that peaked 48 h posttransfection (Fig. 6B). Although both the NES- and NLS-targeted AR112Q proteins were toxic, intracellular localization of the mutant protein did differentially affect other cellular responses as detected by gene expression analysis. Significance analysis of microarrays (35) identified 83 genes that were differentially expressed in a glutamine length-dependent manner by cells transfected with the NES versus NLS constructs (with predicted false discovery of 1.5 genes on microarrays containing 20,000 genes) (supplemental Fig. 2).

To evaluate whether the UPR was similarly activated by NES- and NLS-targeted proteins, HeLa cells were co-transfected with UPRE-luciferase, and reporter gene activation was monitored at several time points posttransfection (Fig. 6C). This analysis revealed time- and glutamine length-dependent luciferase activation in cells transfected with proteins targeted to either the nucleus or cytoplasm. The effects of UPR activation on glutamine length-dependent toxicity were examined by co-transfection with ATF6 and PERK mutants. Significant changes in caspase 3 activity induced by NES AR112Q were observed following co-transfection with the ATF6 DN, ATF6 CA, and PERK DN mutants (Fig. 6D). Although similar trends were observed with NLS AR112Q, a significant change in toxicity was only observed following co-transfection with ATF6 DN (data not shown). These data suggested that the UPR was a more potent modulator of glutamine length-dependent toxicity when the mutant proteins were localized to the cytoplasm.

**DISCUSSION**

We describe the role of the UPR as a modulator of polyglutamine toxicity in cell culture. Our model uses amino-terminal fragments of AR that others and we have shown trigger cell death in a time- and glutamine length-dependent manner. Using this system, we demonstrate glutamine length-dependent UPR activation by increased UPRE-luciferase activity, by phosphorylation of endogenous PERK and nuclear translocation of ATF6. The effects of UPR activation on toxicity were examined by using mutants of the three ER transmembrane proteins that function as proximal sensors of ER stress, ATF6, IRE1, and PERK. Caspase 3 activity was chosen as one measure of toxicity, because UPR-induced cell death is apoptotic, and because an orthologue of mouse caspase 12, an ER stress-induced caspase, has not been identified in humans (22). As a confirmatory, caspase 3-independent cell death assay, dead cells identified with the live/dead assay were visualized and directly counted.

Although prior studies have indicated that proteins with long glutamine tracts activate the UPR (32, 33), the consequences of this activation were not fully characterized. Our data highlight the complex effects this pathway exerts on the survival of cells expressing long glutamine tracts. Although PERK activation promotes death of cells expressing AR112Q, ATF6 activation promotes cell survival. The interdependence of multiple factors leading to cell death often results in only modest effects from single gene loss- or gain-of-function mutations. In this context, the observed effects of the ATF6 and PERK mutants on AR112Q-induced cell death are compatible with a significant biologic role for the UPR as a modulator of polyglutamine toxicity. An important downstream target of ATF6 is BiP, an ER chaperone that binds to the luminal domains of APT6, PERK, and IRE1, thereby preventing their activation (22). This raised the possibility that BiP could mediate the prosurvival effects of ATF6, perhaps through the down-regulation of PERK. However, BiP overexpression does not significantly decrease caspase 3 activation by AR112Q, suggesting that other factors contribute to this prosurvival effect.

We did not observe a significant role for IRE1 as a modulator of AR112Q toxicity. Prior studies have implicated IRE1 as a mediator of the toxicity of a SCA3 fragment containing an expanded glutamine tract through the induction of ASK1 and JNK (33). Here we found that expression of three different IRE1α DN mutants failed to alter AR112Q toxicity. These negative results were obtained despite evidence of IRE1 activation, derived from glutamine length-dependent induction of UPRE-luciferase. Our data suggest that the proapoptotic effects of pathways other than IRE1–ASK1–JNK, including the PERK

**FIG. 5.** Z-VAD-FMK accentuates activation of the UPR by AR112Q. HeLa cells were transfected with AR16Q or AR112Q, and treated with 100 μM Z-VAD-FMK or vehicle control for 48 h. Lysates were evaluated by Western blot for expression of phosphorylated eIF2α (top panel), BiP (middle panel), and β-tubulin (bottom panel).
arm of the UPR, are sufficient to mediate AR112Q toxicity.

Although AR112Q robustly induced the UPR, we also observed a low level UPRE-luciferase activation and toxicity in cells expressing AR16Q. Similarly, other investigators have detected a low level toxicity in cells expressing AR16Q (42). Therefore, it was not surprising that transient overexpression of AR16Q led to low level toxicity in cells expressing AR16Q (42).

Complex effects of UPR activation on cell death and survival are not unique to our system and have been observed by others as well. For instance, the induction of apoptosis by free cholesterol in macrophages is mediated by the UPR (43). Although disruption of PERK signaling in macrophages promotes cholesterol-induced apoptosis, disruption of CHOP, a transcription factor up-regulated in response to PERK activation, attenuates cell death. These findings demonstrate that activation of even a single arm of the UPR may have varied consequences on cell survival. The dominant cellular response may depend on both the stressor and the duration of UPR activation.

Among neurological disorders, UPR activation plays an important role as a modulator of disease severity in Pelizaeus-Merzbacher disease, an X-linked leukodystrophy caused by mutations in the gene encoding proteolipid protein (44). Some pathogenic mutations cause an accumulation of misfolded mutant proteolipid protein in the ER lumen, leading to UPR acti-
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ovation. The UPR has also been implicated in the pathogenesis of several adult onset neurodegenerative disorders associated with protein misfolding and aggregation, including Alzheimer and Parkinson disease as well as the polyglutamine expansion disorders (45). The UPR may similarly impact neurodegeneration in patients with neuroserpin mutations, a disease in which the mutant protein accumulates in the ER lumen (46, 47). Our data demonstrate that the UPR modulates toxicity of expanded glutamine tracts in cell culture. Although these mutant proteins are not known to traffic into the ER lumen, the UPR may be activated in response to proteasome inhibition mediated by their misfolding. Our findings suggest that this pathway may modulate disease severity and may serve as a therapeutic target for the treatment of these disorders.

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