JAZ (Znf346), a SIRT1-interacting Protein, Protects Neurons by Stimulating p21 (WAF/CIP1) Protein Expression*

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Sathi Mallick§ and Santosh R. D’Mello§†

From the Department of Molecular and Cell Biology, University of Texas at Dallas, Richardson, Texas 75080 and the Department of Biological Sciences, Southern Methodist University, Dallas, Texas 75275

**Background:** The mechanisms regulating the survival and death of neurons are poorly understood.

**Results:** JAZ promotes neuronal survival by stimulating the expression of p21 (WAF/CIP1) transcriptionally, thereby inhibiting aberrant cell cycle re-entry.

**Conclusion:** JAZ is a novel regulator of neuronal survival.

**Significance:** Understanding how JAZ protects neurons could lead to the development of novel therapies for neurodegenerative diseases.

SIRT1, a class III histone deacetylase, protects neurons in various models of neurodegenerative diseases. We previously described that neuroprotection by SIRT1 is independent of its catalytic activity. To elucidate how SIRT1 protects neurons, we performed a mass spectrometric screen to find SIRT1-interacting proteins. One of the proteins identified was JAZ (Znf346), a member of a new class of Cys-2–His-2 zinc finger proteins. To investigate the significance of JAZ in the regulation of neuronal survival, we overexpressed it in neurons. We found that JAZ protects cerebellar granule neurons against potassium deprivation-induced death and cortical neurons from death resulting from oxidative stress. JAZ also protects neurons against toxicity induced by mutant huntingtin and mutant ataxin-1 expression. Although expression of endogenous JAZ does not change in neurons primed to die, knockdown of its expression promotes death of otherwise healthy neurons. In contrast to its protective effect in neurons, overexpression of JAZ in different cell lines promotes death. We find that JAZ suppresses cell cycle progression, thereby explaining its contrasting effect in postmitotic neurons versus proliferating cell lines. Although not affecting the expression of several cyclins, overexpression of JAZ stimulates expression of p21 (WAF1/CIP1), a cell cycle inhibitor known to have neuroprotective effects. Results of chromatin immunoprecipitation and transcriptional assays indicate that the stimulatory effect of JAZ on p21 expression is mediated at the transcriptional level. Furthermore, knockdown of p21 expression inhibits the neuroprotective effect of JAZ. Together, our results suggest that JAZ protects neurons by inhibiting cell cycle re-entry through the transcriptional stimulation of p21 expression.

SIRT1, the mammalian homolog of yeast Sir2, is a class III-NAD+-dependent histone deacetylase that protects neurons from death in several tissue culture and *in vivo* models of neurodegenerative disease (1–3). Although neuroprotection by SIRT1 is generally believed to be dependent on its deacetylase activity, we previously reported that SIRT1 could protect neurons by a catalytic activity-independent mechanism (4). We showed that protection by SIRT1 in cultured neurons was not reduced by three separate pharmacological SIRT1 inhibitors and that mutant forms of SIRT1 deficient in catalytic activity were as neuroprotective as wild-type SIRT1 (4). More recently, we have examined the effect of a panel of SIRT1 deletion mutants and found that mutants lacking substantial portions of the catalytic domain of SIRT1 or lacking the essential for Sirt1 activity (ESA) region, recently identified as obligatory for SIRT1 deacetylase activity (5), retain full neuroprotective activity. To gain insight into the catalytic-independent mechanism by which SIRT1 protects neurons, we conducted a screen aimed at identifying SIRT1-interacting proteins in which SIRT1 was overexpressed in HT22 neuroblastoma cells and co-immunoprecipitated proteins identified by mass spectrometric analysis. One of the proteins identified in this screen was JAZ, also referred to as ZnF346.

JAZ (Just Another Zinc finger protein) is the founding member of a new class of C2H2-type zinc finger proteins that was first identified as a gene up-regulated by interleukin-3 deprivation (6). JAZ is expressed widely and relatively highly in most organs, including the brain (6). It is localized primarily in the nucleus, but at least one study has described nucleo-cytoplasmic shuttling (7). There are only a handful of publications on JAZ, and thus its biological functions are not well understood. Although lacking a classical dsRNA-binding domain, JAZ binds dsRNA with high affinity *in vitro* through its C2H2 zinc fingers (6, 8). More recent work has suggested that JAZ mediates cell cycle arrest at the G1 phase (9, 10). Both p53-dependent and -independent mechanisms have been suggested for this inhibitory effect on cell cycle progression (9, 10).

We report that, like SIRT1, JAZ has strong neuroprotective activity, protecting in models where death is not due to pro-
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teinopathic stress as well as in a model of Huntington disease and spinocerebellar ataxia type-1 where accumulation of misfolded protein aggregates triggers neuronal death. We find that JAZ protects neurons by inhibiting cell cycle machinery through the up-regulation of the cyclin-dependent kinase inhibitor protein p21 (WAF1/CIP1). Indeed, knockdown of p21 blocks the neuroprotective effect of JAZ.

EXPERIMENTAL PROCEDURES

Materials—Unless stated otherwise, all of the reagents and cell culture media were obtained from Invitrogen. All chemical reagents were purchased from Sigma. Tissue culture poly-L-lysine was purchased from Trevigen (Gaithersburg, MD). Anti-FLAG antibodies used in this paper were as follows: FLAG (catalog no. sc-104, Santa Cruz Biotechnology, Santa Cruz, CA); HA (Y-11 catalog no. sc-805 and F-7 catalog no. sc-7392, Santa Cruz Biotechnology, Santa Cruz, CA); α-tubulin (TU-02 catalog no. sc-8035, Santa Cruz Biotechnology); GFP (B-2 catalog no. sc-9996a and FL catalog no. sc-8334, Santa Cruz Biotechnology); JAZ (Znf346 catalog no. SAB3500568, Sigma); p21 (catalog no. 556430, BD PharmingenTM); and p27 (catalog no. 8240, BD PharmingenTM), respectively.

Virus Production—Virus was amplified in the HEK293A cell line and purified using CsCl density gradient centrifugation. The final step of the purification process was dialysis using 1 mM HEPES/100 mM NaCl (low potassium, 5 mM) medium (12, 13). Viability of the transfected cells was determined 24 h later by DAPI staining based on the morphology of the cell nuclei. Survival was normalized to GFP in HK condition.

Cortical cultures were obtained from the cerebral cortex of Wistar rats (day 17 or 18 of gestation) using neurobasal media with B27 supplement. Five days after plating, the cultures were transfected using the calcium phosphate method as described previously (13, 14). 8 h after transfection, 1 mM homocysteic acid (HCA) was added for 15–18 h. HCA induces death as a result of oxidative stress as described previously (15–17). HCA stock solution was 100 mM at pH 7.5.

RNA Preparation and RT-PCR—RNA was extracted from cell cultures or cell lines according to the manufacturer’s instructions using TRizol (Invitrogen). cDNA was prepared using the Superscript First Strand Synthesis system for RT-PCR kit (Invitrogen), and PCR was performed with GoTaq Green Master Mix (Promega, Madison, WI). The primers used for PCR amplification were as follows: actin forward, 5′-GGCTGAGGTCGCTCTGGCC-3′, and actin reverse, 5′-CGAAGTTGGAGGTTGCTGAG-3′. The PCR products were fully sequenced before utilization in RT-PCR or Western blot analysis, respectively.

Neuronal Culture, Treatments, and Viability Assay—CGNs were cultured from 7- to 8-day-old Wistar rats and were plated as described previously (11). Transient transfection was performed 5 days after plating for 8 or 24 h (see figure legends) following by switching to HK (high potassium, 25 mM) or LR (low potassium, 5 mM) medium (12, 13). Viability of the transfected cells was determined 24 h later by DAPI staining based on the morphology of the cell nuclei. Survival was normalized to GFP in HK condition.

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mM Na₂EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na₃VO₃, 1 µg/ml leupeptin, and protease inhibitor mixture tablets). The lysates were then kept at −80 °C for at least an hour before proceeding to Western blot analysis. Western blots were performed as described previously (13, 14). Primary antibodies were used in a 1:1000 dilution except JAZ antibody at 1:5000 and tubulin at 1:10,000, and secondary antibodies were used in a 1:10,000 dilution.

Cell Line and Transfection—For viability experiments involving JAZ overexpression, HT22 (mouse hippocampal cell line) and HEK293T (human embryonic kidney cell line) cells were 25–30% at the time of transfection, and viability was quantified 24 h later. For co-immunoprecipitation and other analyses with these cell lines, cells were at 40–50% confluence when transfected.

shRNA Knockdown Experiment—For knockdown analysis of JAZ, five shRNAs were obtained from Sigma (TRCN0000099256, TRCN0000099258, TRCN0000099259, TRCN0000316265, and TRCN0000348955) denoted as JAZ Sh1, JAZ Sh2, JAZ Sh3, JAZ Sh4, and JAZ Sh5, respectively. The pLKO.1-TRC control vector, which contains a non-hairpin 18-bp insert, was used as a transfection control. Five different shRNAs were obtained for p21 knockdown from Sigma (TRCN0000042583, TRCN0000042585, TRCN0000042587, TRCN0000054901, and TRCN0000054902) denoted as ShA, ShB, ShC, ShD, and ShE. The efficiency of the shRNAs was checked in both HEK293T (by suppression of ectopically expressed rat JAZ construct) and HT22 cell line by overexpressing shRNAs for 3 days.

BrdU Assay—BrdU assay was performed in cultures that had been transfected as described previously (18). Briefly, 20 µM BrdU was added to the medium 22 h after transfection for 2 h. Cells were then fixed, washed with 0.5% Triton X-100, and followed by washes with 1 and 2 N HCl. Immunocytochemistry was performed using BrdU antibody (catalog no. B8434, Sigma) at a 1:100 dilution along with another antibody to the tag of the transfected protein. Incubation with secondary antibody was performed the following day before the slides were analyzed by fluorescence microscopy.

Immunoprecipitation—Cell lysates for performing immunoprecipitation were collected as described under “Western Blotting.” An aliquot of the pre-immunoprecipitation whole cell lysate (10% input) was mixed with 6× SDS and prepared for Western blot analysis. The rest of the lysates were subjected to pulldown with the respective antibodies and protein A/G beads. The bead-bound antibodies were allowed to bind to protein at 4 °C overnight with constant stirring followed by washes with 1× cell lysis buffer. The lysate was then subjected to Western blot analysis. To perform endogenous co-immunoprecipitation, 17-day rat brain was collected in 1× RIPA buffer followed by homogenization. 1500 µg of protein was used for pulldown, and 150 µg of protein was used for input.

Image Analysis—ImageJ was used to quantify the immunocytochemistry panels. Area, minimum and maximum gray values, and integrated density (intensity) were selected in the set measurement option. Integrated density was divided by the area of the transfected cells and then normalized to its surrounding cells. For quantification of Western and RT-PCR data, KODAK 1D Image Analysis software was used. The inten-
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Chromatin Immunoprecipitation (ChIP)—ChIP assay was performed as described previously (14). Briefly, transfection was performed in the HEK293T cell line for 40 h. For rat CGNs, 6- to 7-day-old cultures were treated with HK/LK medium for 3 or 6 h. Cells were then fixed with formaldehyde, lysed, and sonicated. The lysates were then subjected to immunoprecipitation with the respective antibody. Reverse cross-linking was performed using elution buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA, pH 8, 1% SDS) at 65 °C. After several rounds of purification (with proteinase and phenol chloroform), the DNA was subjected to PCR analysis using primers designed for the human p21 gene promoter: p21 pro-forward, 5′/H11032-CGCACA-CGGTGTCTCTAAGT-3′, and p21 pro-reverse, 5′/H11032-ACGAACT-TTACTCCACTCCG-3′; p27 promoter primers, p27 pro-forward, 5′/H11032-CTGGGTTAAGGCTGAGCGAA-3′, and p27 pro-reverse, 5′/H11032-TCTGGGTAAAGGCTGAGCGAA-3′; p53 promoter primers, p53 pro-forward, 5′/H11032-CAGGTTTACAACCCGACCTGG-3′, and p53 pro-reverse, 5′/H11032-TGGGTGTAAGGACCCGACCTGG-3′; and cyclin D1 promoter primers, cyclin D1 pro-forward, 5′/H11032-ACCGACTGGTCAAGGATG-3′, and cyclin D1 pro-reverse, 5′/H11032-TATCCGAGCCGAGGATGG-3′. Primers designed to the rat p21 gene promoter are as follows: p21 pro-forward, GCCCTGTGCTTAGGTCATT, and p21 pro-reverse, AAGGAAGGCTCGTGTTAGC.

Luciferase Assay—For transcriptional assays, the luciferase reporter plasmid (WWP-luc) was co-transfected with either GFP or JAZ along with Renilla. The cells were lysed 36 h after transfection, and assays were performed (19, 20) using the Promega Dual-Luciferase® reporter assay system (catalog no. E1910) according to the manufacturer’s instructions. Briefly, the cultures were lysed with 1× lysis buffer provided in the kit. Substrate was added to the lysate and luciferase activity was measured in a luminometer. Stop and Glow reagent was then added to quench the luciferase activity followed by measurement of Renilla activity. Fold induction indicates the ratio of luciferase activity to Renilla activity.

Statistical Analysis—GraphPad Prism 5 software (GraphPad Software, San Diego) was used to generate all graphs used in this paper. Two-tailed Student’s t test was used to perform statistical analysis unless otherwise mentioned, and the results are shown as mean ± S.E. p values of <0.05 were considered as statistically significant. Unless stated otherwise, each experiment was performed in duplicate and repeated at least three times. ≥200 cells were counted for each experiment.

RESULTS

Identification of JAZ as a SIRT1-interacting Protein—We recently performed a screen aimed at identifying SIRT1-interacting proteins by immunoprecipitating ectopically expressed...
SIRT1 in mouse HT22 neuroblastoma cells and identifying proteins that co-immunoprecipitated by mass spectrometry. JAZ was one of several proteins identified in this screen.4 Expression of JAZ in whole brain has previously been reported (6). We extend this observation to show that JAZ is widely expressed in the brain (Fig. 1, A and B). Although JAZ RNA is expressed at similar levels in different brain regions, JAZ protein shows differences suggesting that stability of JAZ is regulated differently in various brain regions, likely due to post-translational mechanisms. Tissue-dependent post-translational modification of JAZ is also suggested by differences in its mobility in extracts from different brain regions (Fig. 1B). We confirmed that JAZ interacts with SIRT1 by co-expressing the two proteins in the HEK293T cell and performing co-immunoprecipitation analysis (Fig. 2A). Indeed, JAZ interacted with SIRT1 more efficiently than HDAC1, a protein known to interact with SIRT1 (21). Results of co-localization studies in CGNs confirmed that JAZ and SIRT1 interact endogenously (Fig. 2B). This was further confirmed by performing co-immunoprecipitation analysis using extracts prepared from rat brain, which showed a robust level of interaction between JAZ and SIRT1 (Fig. 2C).

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FIGURE 4. Suppression of JAZ expression induces death of otherwise healthy neurons. A, whole cell lysates were collected from HEK293T cells transfected with JAZ Sh1, JAZ Sh2, and JAZ Sh3 along with rat JAZ-FLAG construct (7.5:0.5) for 48 h. pLKO1 and water along with rat JAZ-FLAG were used as controls. Western blot analysis was performed using FLAG antibody. ERK serves as a loading control. B, quantification of JAZ protein level in the presence of JAZ shRNAs. Expression levels of JAZ shRNA-transfected cultures was normalized to that of pLKO1-transfected cells. Quantification was performed from three independent experiments. *, p < 0.05, JAZ-transfected cells as compared with control (pLKO1-transfected cells). C, CGNs were co-transfected with pLKO1 (control), JAZ Sh1, JAZ Sh2, or JAZ Sh3 along with GFP (to visualize transfected neurons) in a 6.5:1 ratio for 48 h and then switched to HK for 24 h. Immunocytochemistry was performed using JAZ antibody. D, quantification of JAZ immunoreactivity in pLKO1 and JAZ shRNA-transfected cells. The intensity of signal was normalized to pLKO1-transfected cells. Results are quantified from three independent experiments. One-way analysis of variance test with Bonferroni’s multiple-comparison post-test was used to examine statistical significance. Top asterisks: ***, p < 0.001, JAZ-Sh1-transfected neurons in HK as compared with pLKO1-transfected neurons in HK. Bottom asterisks: ***, p < 0.0001, JAZ Sh1-transfected neurons in HK as compared with pLKO1-transfected neurons in HK. E, whole cell lysates were collected from HT22 cells transfected with pLKO1 (control), JAZ Sh1, and JAZ Sh2 for 72 h. Western blot analysis was performed using JAZ antibody. Tubulin serves as a loading control. F, rat CGNs co-transfected with GFP (to visualize transfected cells) and pLKO1, JAZ Sh1, JAZ Sh2, or JAZ Sh3 in a ratio of 1:6.5 for 48 h and then switched to HK or LK medium for 24 h. Data represent the mean ± S.E. from seven independent experiments. Top asterisks: ***, p < 0.0001, JAZ Sh1-transfected neurons in LK as compared with pLKO1-transfected neurons in LK. *, p < 0.05, JAZ Sh2-transfected neurons as compared with pLKO1-transfected neurons in HK.

4 S. Mallick and S. R. D’Mello, unpublished data.
JAZ Is a Neuroprotective Protein—SIRT1 protects neurons from death in a variety of model systems (2, 22, 23). To investigate whether JAZ also regulates neuronal survival, we used cultured CGNs. CGNs undergo apoptosis when switched from medium containing depolarizing levels of potassium (HK) to medium containing low potassium (LK) (11). We examined the effect of overexpressing JAZ on CGNs treated with HK or LK medium. Although it has no effect on neurons in HK, elevated levels of JAZ prevent LK-induced neuronal death (Fig. 3A).

Additional experiments in which the epitope tag on the ectopically expressed JAZ was switched from FLAG to HA demonstrated that neuroprotection was tag-independent (Fig. 3B). To examine whether the protective effect of JAZ could be seen in paradigms other than LK-treated CGNs, we used cortical neurons treated with HCA. Treatment of cortical neurons with HCA induces death due to oxidative stress (13, 14). JAZ also protected cortical neurons from HCA-induced toxicity (Fig. 3C).

JAZ has been reported to be capable of shuttling between the nucleus and cytoplasm (7). In CGNs, however, ectopically expressed JAZ and the endogenous protein localize to the nucleus (Fig. 3D). Similarly, in cortical neurons JAZ was always in the nucleus (data not shown).

Although overexpression of JAZ could protect neurons from various death-inducing stimuli, it was unclear whether JAZ expression was necessary for the normal survival of neurons. To investigate this issue, we performed shRNA-mediated knock-
down experiments. To identify shRNAs that were effective in suppressing JAZ expression, we tested the efficacy of three commercially available shRNA constructs against JAZ that are designated in this study as Sh1, Sh2, and Sh3. Experiments targeting ectopically expressed JAZ protein in HEK293T cells as well as endogenous protein in HT22 cells demonstrated that Sh1 reduced JAZ expression substantially (Fig. 4, A, B, and E).

Knockdown of JAZ was confirmed in CGNs also using immunocytochemistry (Fig. 4, C and D). In comparison with Sh1, Sh2 had a partial effect of JAZ expression and Sh3 had no discernible effect. Expression of Sh1 promoted death of otherwise healthy CGNs (in HK) and increased the extent of neuronal loss in LK (Fig. 4F). While also reducing survival, the extent of neuronal loss by Sh2 was less than that observed with Sh1, whereas Sh3 had no effect. The correlation of reduced neuronal survival with knockdown efficiency of the three shRNAs indicates that elevated expression of JAZ is necessary for neuronal survival.

Although forced knockdown kills neurons that are normally healthy, it was unclear whether JAZ expression is normally reduced in dying neurons. We investigated this issue in LK-treated CGNs. Neither JAZ mRNA nor protein expression was reduced by LK treatment (Fig. 5, A–D). The same result was found in cortical neurons treated with HCA (Fig. 5, E–G). Taken together with reduced neuronal survival by forced knockdown of JAZ, this finding suggests that the survival-promoting activity of JAZ is regulated by post-translational mechanisms rather than at the level of its expression.

**JAZ Protects Neurons by Inhibiting Cell Cycle Progression through the Stimulation of p21 Expression**—Although promoting the survival of neurons, expression of JAZ in proliferating cell lines results in their death (Fig. 6, A and B). This result is consistent with previous reports describing an inhibitory effect of JAZ on cell cycle progression (9, 10). Aberrant activation of the cell cycle machinery has been implicated in a variety of in vitro and in vivo paradigms of neurodegeneration. Indeed, overexpression of proteins that inhibit cell cycle progression or treatment with pharmacological inhibitors of the cell cycle protects neurons against death induced by a variety of apoptotic stimuli (18, 24–27). It was therefore possible that JAZ protected neurons by the same mechanism by which it killed proliferating cells, i.e. inhibition of the cell cycle. As described previously (9, 10), BrdU incorporation assays conducted in two separate cell lines showed that JAZ inhibits cell cycle progression (Fig. 6, C–E). Although a previous publication has described that this anti-proliferative effect involves interaction of JAZ with E2F1 (10), we were not able to detect such an interaction (data not shown). To gain insight into how JAZ inhibited cell cycle progression, we examined the effect of its expression on various molecules that regulate the cell cycle either positively or negatively. As shown in Fig. 7, no change in expression...
was observed on the expression of cyclins A, B1, D1, and E. Interestingly, JAZ stimulates the expression of the cyclin-dependent kinase inhibitory protein p21 (WAF1/CIP1) but not other negative regulators of the cell cycle, including p15, p27, and p57.

Because p21 has previously been implicated in neuroprotection (15, 28–32), we investigated whether the neuroprotective effect of JAZ was mediated by the stimulation of p21. We first examined whether overexpression of JAZ increases p21 expression in neurons. As shown in Fig. 8, A and B, JAZ overexpression in CGNs results in a robust stimulation of p21 expression. Furthermore, knockdown of JAZ results in the reduction of p21 levels both in the HT22 cell line (Fig. 8C) and in CGNs (Fig. 8, D and E). To investigate whether the effect of JAZ on p21 expression was mediated directly, we examined whether JAZ associates with the p21 gene promoter. Results of ChIP assays indicated that JAZ does bind to the p21 gene promoter. In contrast, binding was not observed with the promoters of the cyclin D1, p27 or p53 genes (Fig. 9A). To verify that JAZ regulates p21 promoter activity, we performed transcriptional assays using a luciferase reporter. As shown in Fig. 9B, expression of JAZ stimulates the transcriptional activity of the p21.
strengthens the conclusions (Fig. 9B) that JAZ stimulates p21 gene transcription in healthy neurons and that its ability to do so is reduced when neurons are primed to die.

If JAZ works by stimulating p21 expression, then direct overexpression of p21 should protect neurons from death without overexpressing JAZ. Indeed, overexpression of p21 in CGNs protects against LK-induced death (Fig. 11A). Furthermore, stimulation of p21 is necessary for the neuroprotective effect of JAZ, knockdown of p21 expression should reduce JAZ-mediated neuroprotection. To perform this experiment, we tested three commercially available p21 shRNA constructs. We found that one of two of these shRNAs, p21-shB and p21-shC, could knock down p21 expression, with shC being more effective (Fig. 11B). Although p21-shA had no effect on the viability of neurons in HK or LK medium, p21-shB and p21-shC reduced survival in HK and potentiated death in LK (Fig. 11C). More importantly, knockdown of p21 expression abrogated the neuroprotective effect of JAZ both in HK- and LK-treated CGNs (Fig. 11C).

JAZ Is Protective in Tissue Culture Models of Neurodegenerative Diseases—HK mimics the survival-promoting effect of neuronal activity during the development of the nervous system. Although this paradigm models developmentally regulated neuronal death, it is not a model of any neurodegenerative disease. To examine whether the neuroprotection by JAZ also extends to disease models, we tested its ability to protect against the toxic effect of mutant huntingtin and mutant ataxin. Neuronal death resulting from mutant-huntingtin (mut-Htt) is frequently used as a cell culture model of Huntington disease. We found that when co-expressed with JAZ, the toxicity of mut-Htt is completely abolished both in HK and LK medium (Fig. 12A). To examine whether this protective effect of JAZ extended to other proteinopathic neurodegenerative disorders, we used mutant ataxin-1 (mut-Atx1). mut-Atx1-induced neuronal death is used as a model of spinocerebellar ataxia type-1. Co-expression of JAZ protects against the neurotoxic effect of mut-Atx1 (Fig. 12B).

DISCUSSION
We describe a new and important regulator of neuronal death, JAZ. Knockdown of JAZ in cultured neurons leads to their death. Although its knockdown kills neurons, JAZ expression is not reduced in neurons primed to undergo apoptosis suggesting that post-translational mechanisms are involved in its ability to maintain neuronal survival. In addition to maintaining neuronal survival, ectopic expression of JAZ protects neurons in different paradigms, including mut-Htt and mut-Atx1 toxicity. JAZ was identified in a mass spectrometric screen for interactors of SIRT1, another protein that has been shown to protect neurons in a number of different studies (22, 23). Interaction between JAZ and SIRT1 is seen in neurons and in whole brain. Although a quantitative comparison cannot be made, the level of JAZ-SIRT1 interaction in whole brain extracts was much higher than when the two proteins were overexpressed in HEK293 cells, suggesting that other factors in
the brain, perhaps involving post-translation of JAZ or involvement of brain-specific proteins enhance JAZ-SIRT1 interaction. The significance of the JAZ-SIRT1 interaction to the neuroprotective activity of either JAZ or SIRT1 is not clear and remains to be elucidated in future studies.

As described previously (9, 10), we find that JAZ inhibits cell cycle progression in proliferating cells. Because abortive cell cycle reentry is widely regarded as a mode by which neurons die both in experimental paradigms and disease states, it is likely that the anti-proliferative effect of JAZ is responsible for its neuroprotective effect. Indeed, we find that JAZ expression increases the expression of p21, a potent cell cycle inhibitor. Several studies have found that elevated p21 levels have a neuroprotective effect (15, 28–32). Results of ChIP and transcriptional assays suggest that the stimulatory effect on p21 expression is transcriptionally mediated through interaction of JAZ with the p21 gene promoter. Increased p21 production in response to ectopic JAZ expression has been described by another laboratory, although in that study the stimulatory effect was believed to be indirect and to involve activation of p53 (9). Although other regulators, such as p53, have been identified as transcriptional regulators of p21, our study identifies JAZ as a novel regulator. Because dysregulation of p21 expression occurs in a variety of pathophysiological conditions, including cancer (33, 34), our finding may have implications to processes in addition to the regulation of neuronal survival and death.

JAZ belongs to a specialized class of Cys-2–His-2 zinc finger proteins that bind preferentially and strongly to double-stranded RNAs (dsRNAs) (35). It has been suggested that JAZ can shuttle between the nucleus and cytoplasm and might be involved in the nucleocytoplasmic shuttling of pre-miRNAs (6, 7). However, both in CGNs and cortical neurons, as well as in the HEK293T and HT22 cell lines, we find JAZ to be strictly localized in the nucleus under all the conditions we used. Thus, although JAZ can shuttle between the nucleus and cytoplasm, it is unlikely that its neuroprotective effect requires dsRNA binding. Other studies have shown that JAZ can interact with proteins, such as p53 and
E2F1, in a manner that is independent of dsRNA binding (9, 10). Although it is possible that the interaction of JAZ with the p21 gene promoter is also mediated through interaction with E2F1, we have not been able to detect interaction between JAZ and E2F1.9

In summary, we describe that JAZ, a poorly studied protein, maintains neuronal survival and protects neurons from death when ectopically expressed. JAZ can protect neurons against toxicity by mutant Htt and mutant Atx1 raising the possibility that finding pharmacological approaches to increase JAZ expression in the brain or gaining a better understanding of how it acts to protect neurons could lead to the identification of novel approaches to treat neurodegenerative diseases like Huntington disease and spinocerebellar ataxia type-1.

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