Praja1 ubiquitin ligase facilitates degradation of polyglutamine proteins and suppresses polyglutamine-mediated toxicity

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Review Timeline:

- Submission Date: 2020-11-24
- Editorial Decision: 2020-12-16
- Revision Received: 2021-06-07
- Accepted: 2021-06-13

Editor-in-Chief: Matthew Welch

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)
RE: Manuscript #E20-11-0747
TITLE: Praja1 ubiquitin ligase facilitates degradation of polyglutamine proteins and suppresses polyglutamine-mediated toxicity

Dear Dr. Mandal:

Thank you for submitting your manuscript entitled "Praja1 ubiquitin ligase facilitates degradation of polyglutamine proteins and suppresses polyglutamine-mediated toxicity" to Molecular Biology of the Cell. I have received reports from two experts in the field that have carefully read your manuscript.

As you will see from the attached reports, both reviewers indicate that the findings are interesting and that the discovery of PJA1 as a new E3 ligase in protein quality control in neurodegenerative diseases is important. Reviewer #2 also appreciates the significant amount of work and the efforts to examine the importance of PJA1 in heterologous systems. Reviewer #2 provides some minor comments that should be addressed. Reviewer #1 expresses more significant concerns, such as the role of PJA1 in protein quality control despite its inability to discriminate between proteins containing short and long polyQ stretches. Some of the concerns can likely be addressed by careful responses and discussions, but some may require additional experimentation. Overall, I feel that carefully addressing the concerns that have been raised would be beneficial and improve the manuscript, and all comments should be addressed in the revision.

Therefore, we would be happy to consider a revised manuscript that satisfies the joint concerns of the reviewers. We look forward to receiving your revised manuscript, together with a letter indicating the changes you have made and the responses to the reviewers' comments.

Sincerely,

James Olzmann
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Mandal,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

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When submitting your revision include a rebuttal letter that details, point-by-point, how the
Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

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In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

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Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

In this manuscript, Ghosh et al. explore the role of the RING-H2 ligase PJA1 in the clearance of the poly-Q repeat containing proteins, ataxin-3 and huntingtin that are implicated in the pathogenesis of spinocerebellar ataxia and huntingtin's disease. The authors identified PJA1 from previously published interactome studies of ataxin-3 and its differential regulation in Huntingtin's disease. They observed a reduction in the expression levels of PJA1 in cells expressing poly-Q repeat containing ataxin-3 (20QT and 80QT) or huntingtin (16QHTT or 83QHTT). They further establish an interaction between PJA1 and ataxin-3 huntingtin with varying polQ expansions and show that an overexpression of PJA1 reduced the aggregates formed by ataxin-3 or huntingtin. Conversely, PJA1 downregulation increased the persistence of these aggregates in the cells. The authors show that the clearance of ataxin-3 aggregates in cells is mediated by ubiquitination which further dictates their degradation by either the proteasome or autophagy. Finally, they provide nice data that PJA1 has evolutionarily conserved roles and is protective against aggregates in a Drosophila rough eye model caused by polyQ toxicity.

The findings are interesting and identifies PJA1 as a new E3 ligase in protein quality control in
neurodegenerative disorders, and the studies in yeast and flies complement the findings in mammalian cells. However, I am perplexed by why PJA1 does not discriminate between short polyQ expansions (that are widely believed to be harmless when expressed in cells) and longer expansions that are cytotoxic. In addition, the impact of PJA1 expression or depletion in many studies does not correlate with the length of polyQ expansion. Thus, I am left wondering how PJA1 identifies toxic aggregates to mediate their clearance. A detailed list of issues that require attention are below.

Fig.1: It is unclear if the authors describe a fold change or a percentage change in the PJA1 transcript levels upon overexpression of 20/80QT (Fig. 1A) or 16/83QHTT (Fig. 1C). Moreover, can the authors provide an explanation for why there is a higher reduction in PJA1 transcript levels upon overexpression of 20QT compared to 80QT?

Fig. 2. The authors show co-localization of PJA1 with 20QT aggregates. Given that shorter expansions do not generally form aggregates, it would informative to count the number of aggregates associated with the overexpression of each construct.

Fig. 3. There are some concerns about appropriate controls in the over-expression studies. It seems the GFP control IP in figure 3A should be used as an indication that PJA1 binding to polyQ proteins in subsequent panels is specific. But I would encourage the authors to repeat the IPs with the GFP control in the same experiment for direct comparison. In Fig. 3B, 3D, and 3I the authors should co-express PJA with the empty vector control. In Fig. 3C the GFP only control is missing altogether. 3B and C: PJA shows the strongest binding to 20QT (as compared to 80QT and 130QF), which is not the most toxic form of Ataxin-3 despite lower expression levels as compared to 80QT. Does this indicate that PJA1 has reduced affinity for the more toxic forms? Or is this because the longer expansions are insoluble and not recovered when lysing cells for IPs? Given the insolubility of longer expansions, and the imaging studies that indicate co-localization of PJA1 to polyQ aggregates, can the authors determine what form of these proteins (soluble or insoluble) PJA1 binds to? Is PJA1 recognizing the polyQ expansion directly? In Fig. 3F, the co-IP band of Ataxin-3 and in Fig. 3G, the PJA1 band are very difficult to see. The figure legend does not describe Fig. 3E at all. Fig. 3D. In the legend, the authors mention that the IP is performed using a GFP antibody, but the figure indicates that the bait is PJA1, which is HA-tagged. It would be useful to clearly label all the epitopes used in the IP studies in this figure.

Fig. 4. The authors suggest that GFP-ataxin-3 and GFP-16Q/83QHTT aggregates are reduced upon overexpression of HA-PJA. Quantification of the number of GFP-aggregates in HA-PJA transfected cells (by co-staining for HA) will provide a direct correlation between PJA1 expression and aggregate clearance. Cell fractionation should be performed to analyze if the reduction in GFP-aggregates is in the soluble or the insoluble fractions. In Figure 4E it is unclear which band corresponds to endogenous Ataxin-3. There are no scale bars in Fig. 4C, and no DAPI stain, thus it is very difficult to interpret these images. Is the green signal the entire cell or an aggregate?

Fig. 5. The authors have used the PJA1 antibody to analyze the levels for endogenous PJA1 in Figure 1. Why was this not used in all the shRNA depletion studies in this figure? The authors show that PJA1 depletion increases the abundance of polyQ proteins. It would be useful here to perform cell fractionation studies to recover soluble and insoluble fractions to assess whether it’s the insoluble pool that increases upon PJA1 depletion. Is the increase in 20Q abundance in shPJA cells cytotoxic or just the increase in longer 80QT and 130QF proteins? In Fig. 5G, there are no scale bars or DAPI stain hence I cannot interpret these images. Is it diffuse (soluble) or aggregated (insoluble) 20QT and 80QT signal that increases in shPJA samples?

Fig. 6. The authors show a dose dependent effect of PJA1 on the stability of 80QT and 130QF. Have the authors looked at the stability of the 20QT, since the same protein seems to be effectively cleared up by PJA1 overexpression in cells? It would also be helpful if the authors can show a protein whose levels stay unaffected upon PJA1 overexpression. In Fig 6G, what is the
rationale for using atg8Δ yeast cells for analyzing the ubiquitination of 20QT, especially since 80QT ubiquitination in 3F is ostensibly in wildtype yeast cells.

Fig. 7. The authors show that PJA1 overexpression leads to a reduction in levels of endogenous ataxin-3. A cycloheximide half-life experiment should be done to assess the stability of endogenous ataxin-3. Does the endogenous ataxin-3 also have a propensity to form aggregates that could be toxic to the cells?

Fig. 8. In 8A, what is the yeast phenotype with only PJA1 expression and does the Delta RING mutant rescue lethality of 80QT? What is the fly eye phenotype when 20QT is expressed? 8D is very difficult to see and the corresponding figure legend should be explained better. What do Alexa fluor 488 and TRITC stain?

This review was carried out by Malavika Raman and Richa Khanna (a post-doctoral fellow in the lab). To promote transparency and to accelerate scientific progress, I no longer participate in anonymous peer review. This review is provided with the understanding that my name will be available to the reviewers and authors.

Reviewer #2 (Remarks to the Author):

Cells manage the accumulation of defective or toxic proteins via several pathways including autophagy and the ubiquitin-proteasome system (UPS). The UPS relies on a series of ubiquitin-conjugating enzymes to mark problematic substrates that then targets them for autophagic collection or degradation via the proteasome. Expanded glutamine repeat proteins are the cause of several neurological and motor diseases in humans, and significant effort has gone into understanding UPS components that may assist in degrading or otherwise ameliorating their toxicity. In this report, the authors mine published interaction data to reveal a new interaction between the Praja1 E3 ubiquitin ligase and polyQ proteins. In a compelling, if unsurprising series of experiments, they demonstrate interaction between Praja1 and both ataxin and Huntington-based polyQ substrates and show that polyQ levels inversely correlate with Praja1 levels. Importantly, they demonstrate that Praja1 acts in this capacity as an E3 enzyme and can do so in the yeast heterologous system. Lastly, using yeast and fly models the authors show that Praja1 indeed acts as a countermeasure to polyQ-induced toxicity and could conceivably be exploited to reduce disease.

This report is a comprehensive investigation into the previously unknown relationship between the Praja1 E3 and polyQ substrates and represents a significant amount of work. Overall the experiments are well-controlled and for the most part presented in a quantitative and rigorous manner. I have modest concerns that should be addressed below:

1) As mentioned above, there are a lot of data in this paper. So much so that it becomes a little confusing to follow the figures due to density. I suggest that the authors move some of the data to online SI to remedy this effect., perhaps by as much as 50%.

2) Fig. 1, plots for panels B and D - shouldn't the Y-axes here be PJA1 levels, not ATA-3 or HTT?

3) Fig. 2, While the descriptive images are nice, this figure requires some quantitative assessment of
protein colocalization.

4) Fig. 3, the immunoblots in many of the panels A-I have extra unlabeled bands showing up. An asterisk is used to identify same in panels G and I but they are all over the place with no asterisks. The authors should specify in all cases which bands are extraneous and identify them, if possible. The result in panel G is almost impossible to discern if I am looking at the right band.

5) In the yeast experiment in Fig. 4 D, the images show either cells full of aggregates, or in the presence of PJA1, cells with diffuse GFP. So it appears that while total 80QT-GFP levels drop modestly, the aggregate clearance is much more dramatically affected. Why is this parameter not quantitated and presented?

6) Fig. 4Biii - Why does the H553 mutation not phenocopy the ΔRING mutation? SHouldn't this substation make PJA1 catalytically dead?

7) A lighter exposure of Fig. 6H, top panel is needed.

8) The autophagy inhibitor 3MA is not explained prior to its use in Fig. 7.

9) The yeast spot tests to show toxicity of 80QT and amelioration are not terribly convincing - the growth defect is modest. Can the authors try a liquid growth curve or some other methodology to try and magnify the differential?

Minor issues:

10) Some odd word choices, but the prerogative of the authors: p. 7 "...we cogitated a physical interaction..."; p. 12 "...ataxin-3 levels were refurbished in..."; p. 15 "...polyQ aggregates voraciously sequester..."

11) cerevisiae misspelled twice on p. 9.
In this manuscript, Ghosh et al. explore the role of the RING-H2 ligase PJA1 in the clearance of the poly-Q repeat containing proteins, ataxin-3 and huntingtin that are implicated in the pathogenesis of spinocerebellar ataxia and huntingtin's disease. The authors identified PJA1 from previously published interactome studies of ataxin-3 and its differential regulation in Huntingtin's disease. They observed a reduction in the expression levels of PJA1 in cells expressing poly-Q repeat containing ataxin-3 (20QT and 80QT) or huntingtin (16QHTT or 83QHTT). They further establish an interaction between PJA1 and ataxin-3 huntingtin with varying polQ expansions and show that an overexpression of PJA1 reduced the aggregates formed by ataxin-3 or huntingtin. Conversely, PJA1 downregulation increased the persistence of these aggregates in the cells. The authors show that the clearance of ataxin-3 aggregates in cells is mediated by ubiquitination which further dictates their degradation by either the proteasome or autophagy. Finally, they provide nice data that PJA1 has evolutionarily conserved roles and is protective against aggregates in a Drosophila rough eye model caused by polyQ toxicity.

The findings are interesting and identifies PJA1 as a new E3 ligase in protein quality control in neurodegenerative disorders, and the studies in yeast and flies complement the findings in mammalian cells. However, I am perplexed by why PJA1 does not discriminate between short polyQ expansions (that are widely believed to be harmless when expressed in cells) and longer expansions that are cytotoxic. In addition, the impact of PJA1 expression or depletion in many studies does not correlate with the length of polyQ expansion. Thus, I am left wondering how PJA1 identifies toxic aggregates to mediate their clearance. A detailed list of issues that require attention are below.

Fig.1: It is unclear if the authors describe a fold change or a percentage change in the PJA1 transcript levels upon overexpression of 20/80QT (Fig. 1A) or 16/83QHTT (Fig. 1C). Moreover, can the authors provide an explanation for why there is a higher reduction in PJA1 transcript levels upon overexpression of 20QT compared to 80QT?

Answer: We have described a fold change in PJA1 transcript levels upon overexpression of ataxin-3 and HTT.

Dysregulation of PJA1 mRNA level by polyQ proteins could be due to the possible transcriptional regulation of PJA1 or other components of the PQC by the polyQ proteins (Saxena and Caroni, 2011; Horvath et al., 2016; Gasset-Rosa et al., 2017). Truncated ataxin-3 is known to translocate to the nucleus and may be involved in transcriptional regulation in a selective manner. We think, that is the case, however establishing this fact is beyond the scope of this manuscript. The higher reduction of PJA1 transcript by 20QT is most likely due to the enhanced expression level of 20QT ataxin-3 in comparison to 80QT as observed in the western blot (Fig 1B) which might lead to differential effects of ataxin-3 on the PJA1 transcript levels. Increased expression levels of 20QT ataxin-3 in the cells might lead to increased polymerization and therefore augmented ability to sequester transcription factors like (NF-κB), CREB binding protein (CBP), TATA box binding protein (TBP), which as a consequence lose their normal cellular functions (Shimohata et al., 2000; Dunah et al., 2002;
Schaffar et al., 2004; Goswami et al., 2006). However, since the difference of PJA1 reduction in presence of 20QT and 80QT is not large, and comparable in its endogenous expression levels, we believe the differential effect might only be due to different expression levels of 20QT and 80QT. Alternatively, ataxin-3 might also directly act as a transcriptional repressor or co-repressor of PJA1. Ergo, higher abundance of 20QT than 80QT, might result in enhanced downregulation of PJA1 mRNA levels in presence of 20QT than of 80QT.

Fig. 2. The authors show co-localization of PJA1 with 20QT aggregates. Given that shorter expansions do not generally form aggregates, it would informative to count the number of aggregates associated with the overexpression of each construct.

Answer: The concern of the reviewer is valid as we know that shorter polyQ expansion generally does not form aggregates. As suggested by Reviewer we have counted the number of aggregates for 20QT. The number of aggregates formed for 20QT is approximately 1.5/100 GFP positive cells, which is much less in comparison to the number of aggregates formed for 80QT and 130QF which are approximately 45 and 15 respectively as shown in Fig.4. The possible explanation could be that truncated ataxin-3 has an accelerated capability to form aggregates. Since the truncated form of ataxin-3 lacks the cysteine protease activity of its J-domain, it loses its neuroprotective function (Warrick et al., 2005; Jung et al., 2009). Additionally, ectopic expression of these wild type truncated ataxin-3 might lead to increased polymerization of the polyQ tracts, eventually leading to formation of aggregates, although the aggregates are much less abundant when compared to the expanded form of ataxin-3.

Fig. 3. There are some concerns about appropriate controls in the over-expression studies. It seems the GFP control IP in figure 3A should be used as an indication that PJA1 binding to polyQ proteins in subsequent panels is specific. But I would encourage the authors to repeat the IPs with the GFP control in the same experiment for direct comparison. In Fig. 3B, 3D, and 3I the authors should co-express PJA with the empty vector control. In Fig. 3C the GFP only control is missing altogether. 3B and C: PJA shows the strongest binding to 20QT (as compared to 80QT and 130QF), which is not be the most toxic form of Ataxin-3 despite lower expression levels as compared to 80QT. Does this indicate that PJA1 has reduced affinity for the more toxic forms? Or is this because the longer expansions are insoluble and not recovered when lysing cells for IPs? Given the insolubility of longer expansions, and the imaging studies that indicate co-localization of PJA1 to polyQ aggregates, can the authors determine what form of these proteins (soluble or insoluble) PJA1 binds to? Is PJA1 recognizing the polyQ expansion directly? In Fig. 3F, the co-IP band of Ataxin-3 and in Fig. 3G, the PJA1 band are very difficult to see. The figure legend does not describe Fig. 3E at all. Fig. 3D. In the legend, the authors mention that the IP is performed using a GFP antibody, but the figure indicates that the bait is PJA1, which is HA-tagged. It would be useful to clearly label all the epitopes used in the IP studies in this figure.

Answer: Taking into consideration the reviewer’s concern we have repeated the CO-IP experiments with appropriate controls in HEK293T cells. We have now replaced the figures with required controls. Additionally, we have included 20Q full length ataxin-3 (20QF) in...
the study (Fig 3A). The data demonstrates that all of the ataxin-3 constructs (20QT, 80QT, 130QF) show similar interaction with PJA1, except 20QF, where we see less interaction, however we do not know the reason at this moment. Also, we cannot say that PJA1 directly interacts with polyQ expansion as we observed the interaction with WT as well as polyQ extended ataxin-3. Molecular chaperones could be a linker in recognizing and subsequently handing the substrate to PJA1 E3 ligase for its ubiquitination. Notably, we have found that PJA1 interacted with DNAJ proteins, DNAJB2 and DNAJB6. Therefore, PJA1 and ataxin-3 interaction can be direct or mediated by molecular chaperones and detailed experimentation is required to establish the fact.
FIGURE 3. PJA1 interacts with normal and polyQ expanded proteins. (A) PJA1 interacts with ataxin-3 proteins. (A) 20Q truncated (20QT), 20Q full length (20QF), 80Q truncated (80QT) and 130Q full length (130QF) ataxin-3 in pEGFP-N1 vector were co-transfected with HA-tagged PJA1 (pCMV-HA-PJA1) in HEK293T cells. 24 hours after transfection, cells were lysed and subjected to immunoprecipitation with anti-GFP antibody. The blots were consecutively probed with anti-HA and anti-GFP antibody for detection of PJA1 and ataxin-3 respectively. The total lysate shows the expression of the respective proteins. (B and C) PJA1 interacts with huntingtin protein. N-terminal fragment of 16Q huntingtin (16QHTT) protein in pEGFP-C1 vector (B) and 83QHTT in pDsRed vector (C) were co-transfected with HA-PJA1 in HEK293T cells. Cells were harvested and processed for immunoprecipitation with anti-GFP (B) and anti-HA (C) antibodies. The blots were successively probed with anti-GFP/anti-DsRed and anti-HA antibodies. (D) PJA1 interacts with endogenous ataxin-3 protein. HA-tagged PJA1 and ΔRING-PJA1 were transfected in HEK293T cells. 24 hours after transfection, cells were lysed and subjected to immunoprecipitation using anti-SCA3 antibody. The blots were consecutively probed with respective antibodies. The total lysate shows the expression of the respective proteins. GAPDH is the loading control for all the experiments. Results are representative of three independent experiments.

For better clarity of the new Figure 3, we have moved few Co-IP figures, mainly PJA1-Ataxin-3 interaction by immunoprecipitating PJA1 in HEK293T cells and in Neuro2A cells in the supplemental material (Fig. S1). Also, we have replaced the figure to show that endogenous ataxin-3 interacts with PJA1 by immunoprecipitating ataxin-3 in HA-PJA1 over-expression condition (Fig. 3D) and the mistake in the legend was taken care of.
FIGURE S1. PJA1 interacts with normal and polyQ expanded proteins. (A, D-E) 20Q truncated (20QT), 80Q truncated (80QT) and 130Q full length (130QF) ataxin-3 in pEGFP-N1 vector were co-transfected with HA-tagged PJA1 (pCMV-HA-PJA1) in HEK293T cells (A) and Neuro2A cells (D and E) respectively. 24 hours after transfection, cells were lysed and subjected to immunoprecipitation either with anti-HA antibody (A and D) or with anti-GFP antibody (E). The blots were consecutively probed with anti-GFP and anti-HA antibody for detection of ataxin-3 and PJA1 respectively. The total lysate shows the expression of the respective proteins. (B) PJA1 interacts with huntingtin protein. N-terminal fragment of 16Q huntingtin (16QHTT) protein in pEGFP-C1 vector was co-transfected with HA-PJA1 in HEK293T cells. Cells were harvested and processed for immunoprecipitation with anti-HA antibody. The blots were successively probed with anti-GFP and anti-HA antibody. (C) PJA1 does not interact with GFP protein. GFP empty vector (EV) and HA-PJA1 constructs were co-transfected in HEK293T cells. Cell lysate was immunoprecipitated with either anti-GFP or anti-HA antibody followed by western blotting and probing with anti-HA and anti-GFP antibody respectively. NT and EV represent non-transfected cells and empty vector respectively.
Regarding the PJA1 binding to the soluble or insoluble form of ataxin-3, we have seen in our experimental set up (24 hours after transfection, since PJA1 is a short-lived protein) that polyQ expanded ataxin-3, 80QT is soluble. So, the chances of differential association of PJA1 with 20QT or 80QT ataxin-3 due to solubility are meek.

**Solubility assay of 80QT ataxin-3.** HA-tagged PJA1 and the corresponding empty vector were transfected with 80QT ataxin-3 into HEK293T cells. 24 hours after transfection, the cells were lysed with RIPA Buffer, centrifuged at 3000 rpm (total) followed by fractioning the lysate by centrifugation at 13,000 rpm into soluble and insoluble parts. The corresponding lysates were then subjected to immunoblotting with anti-GFP and anti-HA antibodies. GAPDH was the loading control.

**Fig. 4.** The authors suggest that GFP-ataxin-3 and GFP-16Q/83QHTT aggregates are reduced upon overexpression of HA-PJA. Quantification of the number of GFP-aggregates in HA-PJA transfected cells (by co-staining for HA) will provide a direct correlation between PJA1 expression and aggregate clearance. Cell fractionation should be performed to analyze if the reduction in GFP-aggregates is in the soluble or the insoluble fractions. In Figure 4E it is unclear which band corresponds to endogenous Ataxin-3. There are no scale bars in Fig. 4C, and no DAPI stain, thus it is very difficult to interpret these images. Is the green signal the entire cell or an aggregate?

**Answer:** We accept the reviewer’s point that aggregates in the cells coexpressing atxn3 and PJA1 be counted for direct correlation between PJA1 expression and aggregate clearance. However, the fluorescence microscopy experiments were repeated several times for its validity, and the data has been collected from more than 50 fields for each set. The expression of PJA1 had been checked each time. The percentage of reduction in the number of 80QT aggregates/100 GFP positive cells in presence of PJA1 have been identical in almost every field, which confirms the data reported in our study.

As these experiments are performed 24 hours after transfection, 80QT ataxin-3 remains in soluble form, as shown above. The solubility assay shows that reduction of ataxin-3 is from its soluble form.

In Figure 4E, the lower band corresponds to endogenous ataxin-3 (45KD), although whenever probed with Anti-SCA3 antibody (Merck), two bands are obtained.

We have included the scale bars in Figure 4C.

The HTT aggregates are mostly cytoplasmic. The 16Q HTT did not form any aggregates, and the green signal is diffused throughout the cytoplasm.
Fig. 5. The authors have used the PJA1 antibody to analyze the levels for endogenous PJA1 in Figure 1. Why was this not used in all the shRNA depletion studies in this figure? The authors show that PJA1 depletion increases the abundance of polyQ proteins. It would be useful here to perform cell fractionation studies to recover soluble and insoluble fractions to assess whether it's the insoluble pool that increases upon PJA1 depletion. Is the increase in 20Q abundance in shPJA cells cytotoxic or just the increase in longer 80QT and 130QF proteins? In Fig. 5G, there are no scale bars or DAPI stain hence I cannot interpret these images. Is it diffuse (soluble) or aggregated (insoluble) 20QT and 80QT signal that increases in shPJA samples?

Answer: We completely agree with the reviewer. However, inconsistency in detection of endogenous PJA1 with available PJA1 antibody (Proteintech or Genetex) compelled us to validate efficacy of si/shRNA targeted towards PJA1 using RT-PCR. Even PJA1 antibody obtained from Proteintech (which has few citations) failed to function properly in our experimental setting, as a result of which we decided to validate our experiments with RT-PCR.

We have observed that expression of 80QT ataxin-3 remains in the soluble form (shown above) since we have expressed it only for 24 hours to see the effect of PJA1. Similar results were found when PJA1 was silenced with si/sh RNA. We see that PJA1 depletion in cells leads to increase in ataxin-3 levels when cells are lysed with RIPA buffer followed by fractionation either at 3000 rpm to get total protein (soluble+ insoluble) or directly at 13,000 rpm to obtain soluble fraction. Thus, PJA1 facilitates clearance of soluble ataxin-3. We also tried to see whether PJA1 can clear 80QT aggregates in yeast system, by expressing 80QT from GAL promoter for a considerable time, then shutting off 80QT expression followed by PJA1 expression from CUP promoter. In this set-up, we were unable to see 80QT clearance by PJA1 which suggests that PJA1 may not clear ataxin-3 in its aggregated form but rather act before it forms aggregates i.e., in its monomeric or oligomeric forms. This indicates that PJA1 is as E3 ligase for WT as well as expanded ataxin-3 protein and not specific for misfolded polyQ expanded ataxin-3.

Previous reports have established that WT ataxin-3 is not cytotoxic, thus increase in 20QT ataxin-3 level upon PJA1 depletion may not be cytotoxic. However, increase in the polyQ expanded forms in the cells (by ectopic expression and/or by siRNA mediated depletion of a specific E3 ligase) is supposed to render the cells toxic, as reported by (Chhangani and Mishra, 2013; Chhangani et al., 2014a; Chhangani et al., 2014b). We had tried to check whether cytotoxicity of 80QT ataxin-3 upon transiently transfecting ataxin-3 construct could be rescued by PJA1 by performing MTT in mammalian cells, but the results were not consistent enough to be reported. Hence, we were unable to perform these experiments by depleting PJA1 in mammalian cells.

In Fig. 5G, we have incorporated the scale bars. In shPJA1 N2A cells, there is overall increase in diffused fluorescence of 20QT ataxin-3, whereas for 80QT, both the overall intensity as well as the number of aggregates increases.
Fig. 6. The authors show a dose dependent effect of PJA1 on the stability of 80QT and 130QF. Have the authors looked at the stability of the 20QT, since the same protein seems to be effectively cleared up by PJA1 overexpression in cells? It would also be helpful if the authors can show a protein whose levels stay unaffected upon PJA1 overexpression. In Fig 6G, what is the rationale for using atg8Δ yeast cells for analyzing the ubiquitination of 20QT, especially since 80QT ubiquitination in 3F is ostensibly in wildtype yeast cells.

Answer: As suggested by the reviewer, dose dependent effect of PJA1 on 20QT was performed which displays similar reduction of 20QT upon PJA1 expression as shown below. However, we had not incorporated this in the main figure.

![Image of western blot analysis showing dose dependent effect of PJA1 on 20QT and 80QT stability](image)

**PJA1 promotes degradation of 20QT ataxin-3 protein.** Neuro2A cells were transiently transfected with 20QT ataxin-3 with varying concentrations of PJA1 as indicated. 24 hours after transfection, ataxin-3 level was detected in cell lysates by western blotting with anti-GFP antibody. GAPDH was used as loading controls.

We had checked the role of PJA1 on α-synuclein protein. To our surprise, α-synuclein levels were unaltered in presence of PJA1.

![Image of western blot analysis showing no change in α-synuclein levels upon PJA1 overexpression](image)

**PJA1 does not clear α-synuclein.** HA-tagged PJA1 and the corresponding empty vector were transfected with GFP tagged α-synuclein into HEK293T cells. 24 hours after transfection, the cells were harvested and the lysates were subjected to immunoblotting with anti-GFP and anti-HA antibodies. GAPDH was the loading control.

We understand the reviewer’s point. We wanted to show that PJA1 not only ubiquitinates polyQ extended ataxin-3 protein for their degradation, but also acts on wild type ataxin-3. To show the ubiquitination of 20QT effectively we have used atg8Δ knock out yeast strain since we have seen that 20QT was stabilized in atg8 knock out system (Fig 7D).
Fig. 7. The authors show that PJA1 overexpression leads to a reduction in levels of endogenous ataxin-3. A cycloheximide half-life experiment should be done to assess the stability of endogenous ataxin-3. Does the endogenous ataxin-3 also have a propensity to form aggregates that could be toxic to the cells?

**Answer:** As we have mentioned in the manuscript, PJA1 is a short-lived protein (Zoabi *et al.*, 2011) in comparison to ataxin-3 as shown below (panel A). As a result, we did not perform cycloheximide chase of ataxin-3 in mammalian cells upon expression of PJA1. In accordance with the reviewer’s suggestion, we have performed cycloheximide chase of endogenous ataxin-3 with/without expression of PJA1 (Panel B). We observed that ataxin-3 level is decreased in presence of PJA1 at the beginning of the chase time and becomes steady afterwards as PJA1 level reduced with time.

Cycloheximide chase of PJA1 (A) and endo ataxin-3 (B) was performed by transiently transfecting HA-PJA1 in HEK293T cells as described in method section. 10μg/ml of cycloheximide was used to stop translation and chased for indicated time. Ataxin-3 and PJA1 levels were detected by western blotting with anti-SCA3 antibody and anti-HA antibodies respectively. GAPDH acts as the loading control. The band intensities from (A) were quantified using imageJ software and plotted in the adjacent graph.

Endogenous ataxin-3 is theoretically not supposed to form toxic aggregates (Paulson *et al.*, 1997).

Fig. 8. In 8A, what is the yeast phenotype with only PJA1 expression and does the Delta RING mutant rescue lethality of 80QT? What is the fly eye phenotype when 20QT is expressed? 8D is very difficult to see and the corresponding figure legend should be explained better. What do Alexa fluor 488 and TRITC stain?

**Answer:** As suggested by the reviewer, we have performed growth assay with only PJA1 and ΔRING-PJA1 to check the yeast phenotype (Panel A). PJA1 was expressed with CUP promoter by induction with copper. Similar growth pattern was observed when empty vector (EV), only PJA1 and ΔRING-PJA1 were over-expressed in yeast. We also tested whether RING deleted PJA1 can suppress the aggregation of 80QT and also its toxicity in yeast. We
see that RING-deleted PJA1 is unable to suppress 80QT aggregation, reduction of 80QT protein level (Panel C) and 80QT mediated toxicity (Panel B).

**ΔRING-PJA1 is unable to rescue toxicity caused by 80QT.** (A) Expression of PJA1 or ΔRING-PJA1 cloned in p426CUP vector do not affect yeast growth. (B) Yeast cells harbouring p315GAL 80QT ataxin-3 alone, and with p426CUP-ΔRING-PJA1 were grown overnight in 2% raffinose. ΔRING PJA1 is induced with 300μM copper for 2 hours followed by induction of ataxin-3 with 2% galactose. The cultures were serially diluted and spotted on to either YPD or YP with 2% galactose and 100μM copper. The plates were incubated at 30°C. Growth of yeast cells were observed after 48 hours. (C) PJA1 expression was promoted by 300μM copper for 2 hours prior to induction of ataxin-3 with 2% galactose for 6 hours. The cells were then subjected to fluorescence microscopy. The same cells were processed for immunoblotting with anti-GFP and anti-HA for detection of ataxin-3 and PJA1. PGK1 indicates loading control.

WT ataxin-3 overexpressed transgenic fly is non-pathogenic. Since we were tracking the role of PJA1 in modulating pathogenesis of mutant ataxin-3, we had not performed our experiments with a WT ataxin-3 transgenic fly. However, taking into consideration that, the phenotype of a GAL4-Rh1 fly has similar eye morphology to a WT ataxin-3 transgenic fly, we have overexpressed PJA1 alone (GAL-Rh1-PJA1), and found that there were no morphological differences when compared to the control GAL4-Rh1 fly. Reperforming these experiments with a WT ataxin-3 transgenic fly is difficult, since the flies need to be procured from Bloomington Drosophila stock centre, which is beyond the scope at this moment.

8Di and Dii show the confocal microscopy images of CsWT and Rh1-GAL4 fly eye sections which have been immunostained with anti-HA (78QT ataxin-3) and anti-PJA1 antibody. Since both the flies neither have ataxin-3 nor PJA1, the retinal sections stain negative for these proteins and hence were taken as controls. We have changed the figure legend and explained it better.

Alexa Fluor 488 stains for ataxin-3 and TRITC for PJA1.
Reviewer #2 (Remarks to the Author):

Cells manage the accumulation of defective or toxic proteins via several pathways including autophagy and the ubiquitin-proteasome system (UPS). The UPS relies on a series of ubiquitin-conjugating enzymes to mark problematic substrates that then targets them for autophagic collection or degradation via the proteasome. Expanded glutamine repeat proteins are the cause of several neurological and motor diseases in humans, and significant effort has gone into understanding UPS components that may assist in degrading or otherwise ameliorating their toxicity. In this report, the authors mine published interaction data to reveal a new interaction between the Praja1 E3 ubiquitin ligase and polyQ proteins. In a compelling, if unsurprising series of experiments, they demonstrate interaction between Praja1 and both ataxin and Huntington-based polyQ substrates and show that polyQ levels inversely correlate with Praja1 levels. Importantly, they demonstrate that Praja1 acts in this capacity as an E3 enzyme and can do so in the yeast heterologous system. Lastly, using yeast and fly models the authors show that Praja1 indeed acts as a countermeasure to polyQ-induced toxicity and could conceivably be exploited to reduce disease.

This report is a comprehensive investigation into the previously unknown relationship between the Praja1 E3 and polyQ substrates and represents a significant amount of work. Overall the experiments are well-controlled and for the most part presented in a quantitative and rigorous manner. I have modest concerns that should be addressed below:

1) As mentioned above, there are a lot of data in this paper. So much so that it becomes a little confusing to follow the figures due to density. I suggest that the authors move some of the data to online SI to remedy this effect, perhaps by as much as 50%.

Answer: As suggested by the reviewer, we have now shifted co-IP data from Figure 3 and yeast data from Figure 4 to supplemental section for better clarity of the figures.

2) Fig. 1, plots for panels B and D - shouldn't the Y-axes here be PJA1 levels, not ATA-3 or HTT?

Answer: We thank the reviewer for pointing out the unintentional mistake. We have corrected the mistake in the figure.

3) Fig. 2, While the descriptive images are nice, this figure requires some quantitative assessment of protein colocalization.

Answer: We have now included Pearson’s correlation coefficient values of the ataxin-3 and PJA1 colocalization, which quantifies the extent of colocalization between two proteins in Figure 2F.
FIGURE 2F. Pearson’s Correlation Coefficients for colocalization between polyQ proteins and PJA1. The plot depicts Pearson’s correlation coefficients for colocalization analysis. The Rp values for 20QT and PJA1 (0.78, IQR 0.6 – 0.8), 80QT and PJA1 (0.77, IQR 0.62 – 0.95), 130QF and PJA1 (0.86, IQR 0.67 – 0.94), 83QHTT and PJA1 (0.88, IQR 0.62-0.95), 80QT and DsRed (0.46, IQR 0.43-0.53) and 83QHTT and GFP (0.36, IQR 0.20-0.52) indicate the extent of colocalization of polyQ proteins and PJA1 and shows that PJA1 and polyQ proteins significantly colocalized with each other.

4) Fig. 3, the immunoblots in many of the panels A-I have extra unlabeled bands showing up. An asterisk is used to identify same in panels G and I but they are all over the place with no asterisks. The authors should specify in all cases which bands are extraneous and identify them, if possible. The result in panel G is almost impossible to discern if I am looking at the right band.

Answer: Yes, we understand the reviewer’s concern. We have reperformed the Co-IP experiments and replaced the figures with appropriate controls.

5) In the yeast experiment in Fig. 4 D, the images show either cells full of aggregates, or in the presence of PJA1, cells with diffuse GFP. So it appears that while total 80QT-GFP levels drop modestly, the aggregate clearance is much more dramatically affected. Why is this parameter not quantitated and presented?

Answer: We have quantified the number of aggregates and included in the figure. However, we have moved the figure in supplemental material as Figure S2 to reduce the number of figures as per reviewer’s suggestion.
FIGURE S2. PJA1 reduces ataxin-3 aggregation in yeast system. (A-B) 80QT ataxin-3 and PJA1 were cloned into yeast inducible vectors under galactose and copper promoters respectively. PJA1 expression was promoted by 300µM copper for 2 hours prior to induction of ataxin-3 with 2% galactose for 6 hours. The cells were then subjected to fluorescence microscopy (A). Aggregates were counted per 100 GFP positive cells from 10 fields randomly chosen and plotted in a bar graph. Values are the mean ± S.D. Significance was calculated by t-test, p< 0.001 (***). The same cells were processed for immunoblotting with anti-GFP and anti-HA for detection of ataxin-3 and PJA1 (B). PGK1 indicates loading control. The bands were then quantified, normalized with PGK1 and plotted as bar diagram. Error bar represents SD of three independent experiments. Significance was calculated by t-test, p<0.05.

6) Fig. 4Biii - Why does the H553 mutation not phenocopy the ∆RING mutation? Shouldn't this substitution make PJA1 catalytically dead?

Answer: The reviewer is correct to point out the concern. PJA1 is a RING-H2 ubiquitin ligase. The RING domain of a RING-H2 ligase, consists of six cysteine and two histidine residues which are stringently oriented to coordinate two Zinc atoms (Borden and Freemont, 1996). Though a single substitution of any of these conserved residues should abolish the ubiquitin ligase activity, studies show that simultaneous substitutions of either C1+C2 or C1+C2+C3 or C6+C7 are required to compromise ligase activity of certain E3 ligases (Garcia-Barcena et al., 2020). Interestingly, a recent study has also established that even simultaneous substitution of four Zn coordinating residues of TRIM27 does not completely render it inactive (Zaman et al., 2013). Since we have mutated a single conserved histidine residue to serine it is possible that it might retain some of its catalytic activity as also shown for RING E3 ligase UBR1 where mutation of single cysteine residue only reduced the efficiency of its catalytic activity but did not completely abolish its activity (Nillegoda et al., 2010).
7) A lighter exposure of Fig. 6H, top panel is needed.

Answer: We have included a lighter exposure of Fig. 6H.

8) The autophagy inhibitor 3MA is not explained prior to its use in Fig. 7.

Answer: We have included a description of usage of 3MA now in the modified manuscript.

9) The yeast spot tests to show toxicity of 80QT and amelioration are not terribly convincing - the growth defect is modest. Can the authors try a liquid growth curve or some other methodology to try and magnify the differential?

Answer: Yes, the reviewer is right to point out a modest growth defect of 80QT. We have repeated it, and the growth defect is not extreme enough. To confirm, the yeast data, we have also performed MTT assay in mammalian system. However, due to transient transfection of ataxin-3 and PJA1, the effects are not consistent enough to report it. Nevertheless, we have shown the data obtained below.

Minor issues:

10) Some odd word choices, but the prerogative of the authors: p. 7 "...we cogitated a physical interaction..."; p. 12 "...ataxin-3 levels were refurbished in..."; p. 15 "...polyQ aggregates voraciously sequester..."

Answer: We have substituted the words pointed out by the reviewers to, cogitated to ‘hypothesized’, refurbished to ‘restored’ and omitted the word ‘voraciously’.

11) cerevisiae misspelled twice on p. 9.

Answer: We have corrected cerevissiae to ‘cerevisiae’.
References

Borden, K.L., and Freemont, P.S. (1996). The RING finger domain: a recent example of a sequence-structure family. Curr Opin Struct Biol 6, 395-401.

Chhangani, D., and Mishra, A. (2013). Mahogunin ring finger-1 (MGRN1) suppresses chaperone-associated misfolded protein aggregation and toxicity. Sci Rep 3, 1972.

Chhangani, D., Nukina, N., Kurosawa, M., Amanullah, A., Joshi, V., Upadhyay, A., and Mishra, A. (2014a). Mahogunin ring finger 1 suppresses misfolded polyglutamine aggregation and cytotoxicity. Biochim Biophys Acta 1842, 1472-1484.

Chhangani, D., Upadhyay, A., Amanullah, A., Joshi, V., and Mishra, A. (2014b). Ubiquitin ligase ITCH recruitment suppresses the aggregation and cellular toxicity of cytoplasmic misfolded proteins. Sci Rep 4, 5077.

Dunah, A.W., Jeong, H., Griffin, A., Kim, Y.M., Standaert, D.G., Hersch, S.M., Mouradian, M.M., Young, A.B., Tanese, N., and Krainc, D. (2002). Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science 296, 2238-2243.

Garcia-Barcena, C., Osinalde, N., Ramirez, J., and Mayor, U. (2020). How to Inactivate Human Ubiquitin E3 Ligases by Mutation. Front Cell Dev Biol 8, 39.

Gasset-Rosa, F., Chillon-Marinas, C., Goginashvili, A., Atwal, R.S., Artates, J.W., Tabet, R., Wheeler, V.C., Bang, A.G., Cleveland, D.W., and Lagier-Tourenne, C. (2017). Polyglutamine-Expanded Huntingtin Exacerbates Age-Related Disruption of Nuclear Integrity and Nucleocytoplasmic Transport. Neuron 94, 48-57 e44.

Goswami, A., Dikshit, P., Mishra, A., Nukina, N., and Jana, N.R. (2006). Expression of expanded polyglutamine proteins suppresses the activation of transcription factor NFkappaB. J Biol Chem 281, 37017-37024.

Horvath, S., Langfelder, P., Kwak, S., Aaronson, J., Rosinski, J., Vogt, T.F., Eszes, M., Faull, R.L., Curtis, M.A., Waldvogel, H.J., Choi, O.W., Tung, S., Vinters, H.V., Coppola, G., and Yang, X.W. (2016). Huntington's disease accelerates epigenetic aging of human brain and disrupts DNA methylation levels. Aging (Albany NY) 8, 1485-1512.

Jung, J., Xu, K., Lessing, D., and Bonini, N.M. (2009). Preventing Ataxin-3 protein cleavage mitigates degeneration in a Drosophila model of SCA3. Hum Mol Genet 18, 4843-4852.

Nillegoda, N.B., Theodoraki, M.A., Mandal, A.K., Mayo, K.J., Ren, H.Y., Sultana, R., Wu, K., Johnson, J., Cyr, D.M., and Caplan, A.J. (2010). Ubr1 and Ubr2 function in a quality control pathway for degradation of unfolded cytosolic proteins. Mol Biol Cell 21, 2102-2116.

Paulson, H.L., Perez, M.K., Trottier, Y., Trojanowski, J.Q., Subramony, S.H., Das, S.S., Vig, P., Mandel, J.L., Fischbeck, K.H., and Pittman, R.N. (1997). Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. Neuron 19, 333-344.

Saxena, S., and Caroni, P. (2011). Selective neuronal vulnerability in neurodegenerative diseases: from stressor thresholds to degeneration. Neuron 71, 35-48.

Schaffar, G., Breuer, P., Boteva, R., Behrends, C., Tzvetkov, N., Strippel, N., Sakahira, H., Siegers, K., Hayer-Hartl, M., and Hartl, F.U. (2004). Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. Mol Cell 15, 95-105.
Shimohata, T., Nakajima, T., Yamada, M., Uchida, C., Onodera, O., Naruse, S., Kimura, T., Koide, R., Nozaki, K., Sano, Y., Ishiguro, H., Sakoe, K., Ooshima, T., Sato, A., Ikeuchi, T., Oyake, M., Sato, T., Aoyagi, Y., Hozumi, I., Nagatsu, T., Takiyama, Y., Nishizawa, M., Goto, J., Kanazawa, I., Davidson, I., Tanese, N., Takahashi, H., and Tsuji, S. (2000). Expanded polyglutamine stretches interact with TAFII130, interfering with CREB-dependent transcription. Nat Genet 26, 29-36.

Warrick, J.M., Morabito, L.M., Bilen, J., Gordesky-Gold, B., Faust, L.Z., Paulson, H.L., and Bonini, N.M. (2005). Ataxin-3 suppresses polyglutamine neurodegeneration in Drosophila by a ubiquitin-associated mechanism. Mol Cell 18, 37-48.

Zaman, M.M., Nomura, T., Takagi, T., Okamura, T., Jin, W., Shinagawa, T., Tanaka, Y., and Ishii, S. (2013). Ubiquitination-deubiquitination by the TRIM27-USP7 complex regulates tumor necrosis factor alpha-induced apoptosis. Mol Cell Biol 33, 4971-4984.

Zoabi, M., Sadeh, R., de Bie, P., Marquez, V.E., and Ciechanover, A. (2011). PRAJA1 is a ubiquitin ligase for the polycomb repressive complex 2 proteins. Biochem Biophys Res Commun 408, 393-398.
Dear Dr. Mandal:

Thank you for submitting the revised version of your manuscript "Praja1 ubiquitin ligase facilitates degradation of polyglutamine proteins and suppresses polyglutamine-mediated toxicity".

After reading your revised manuscript and your rebuttal letter, I have concluded that the new data and changes you have added to the manuscript address the reviewers' concerns and significantly improve the paper. I am pleased to report that your manuscript is now acceptable for publication in Molecular Biology of the Cell without further modifications. Thank you for submitting this interesting paper to the journal.

Sincerely,
James Olzmann
Monitoring Editor
Molecular Biology of the Cell

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