In at least nine inherited diseases polyglutamine expansions cause neurodegeneration associated with protein misfolding and the formation of ubiquitin-conjugated aggregates. Although expanded polyglutamine triggers disease, functional properties of host polyglutamine proteins also must influence pathogenesis. Using complementary in vitro and cell-based approaches we establish that the polyglutamine disease protein, ataxin-3, is a poly-ubiquitin-binding protein. In stably transfected neural cell lines, normal and expanded ataxin-3 both co-precipitate with poly-ubiquitinated proteins that accumulate when the proteasome is inhibited. In vitro pull-down assays show that this reflects direct interactions between ataxin-3 and ubiquitin conjugates; ataxin-3 binds K48-linked tetra-ubiquitin but not di-ubiquitin or mono-ubiquitin. Further studies with domain-deleted and site-directed mutants map tetra-ubiquitin binding to ubiquitin interaction motifs situated near the polyglutamine domain. In surface plasmon resonance binding analyses, normal and expanded ataxin-3 display similar submicromolar dissociation constants for tetra-ubiquitin. Binding kinetics, however, are markedly influenced by the surrounding protein context; ataxin-3 that lacks the highly conserved, amino-terminal josephin domain shows significantly faster association and dissociation rates for tetra-ubiquitin binding. Our results establish ataxin-3 as a poly-ubiquitin-binding protein, thereby linking its normal function to protein surveillance pathways already implicated in polyglutamine pathogenesis.

The inherited polyglutamine (polyQ) diseases illustrate a recurring theme in neurodegenerative diseases, perturbations in normal protein homeostasis as a central element of pathogenesis. Most acquired and inherited neurodegenerative diseases are characterized by abnormal protein folding, processing, and/or aggregation (1–3). This is particularly well documented for the polyQ diseases, a group of at least nine dominantly inherited disorders in which expanded CAG repeat mutations encode abnormally long polyQ tracts in the various disease proteins (1, 4). It is widely agreed that expanded polyQ triggers disease, but precisely how it causes neuronal dysfunction and cell death remains elusive. Expanded polyQ is prone to misfold and aggregate, forming intraneuronal, ubiquitin-positive inclusions. These inclusions also sequester components of the proteasome and other proteins, including molecular chaperones. Studies from a wide range of cell and animal models implicate the quality control machinery of the cell, notably chaperones and the ubiquitin-proteasome pathway, in disease pathogenesis (1, 5–7).

Although it is clear that expanded polyQ triggers disease, host protein context must also contribute to pathogenesis in unique ways for each disease (8). Other than sharing polyQ, the nine disease proteins are entirely unrelated and likely serve different functions in the cell. For example, the polyQ disorders Huntington disease and spinocerebellar ataxia 3 (also known as Machado-Joseph disease, or SCA3/MJD) manifest with very distinct clinical and pathological features. Most likely this reflects the fact that the two gene products, huntingtin and ataxin-3, bind different protein partners and function in distinct cellular pathways. Accordingly, a full understanding of each polyQ disease will require that the functional properties of each host protein also be defined.

In the current study we explore the function of the SCA3/MJD disease protein, ataxin-3 (9). The smallest of the polyQ disease proteins, ataxin-3, resides both in the cytoplasm and nucleus, where it binds the nuclear matrix (10–13). Despite in vitro studies that have successfully modeled ataxin-3 protein folding (14), relatively little is known about its functional properties. Some studies suggest that ataxin-3 negatively regulates gene expression events (15, 16), perhaps by inhibiting histone deacetyltransferases, but there is no direct evidence that ataxin-3 functions as a transcription factor or cofactor. An important clue to its possible function surfaced in a recent bioinformatics study, revealing that ataxin-3 contains predicted ubiquitin interaction motifs, or UIMs (17). These short motifs have been shown to mediate ubiquitin binding by UIM-containing proteins, many of which function in nonproteasomal ubiquitin pathways such as vesicular trafficking (18–20).

The existence of predicted UIMs in ataxin-3 is especially intriguing because ubiquitin-proteasome pathways have already been implicated in SCA3/MJD and other polyQ diseases (7, 21, 22). Moreover, ataxin-3 has a property unique among polyQ disease proteins in that the normal ataxin-3 protein localizes to neuronal protein inclusions known as Neurotes bodies, ubiquitin-rich aggregates found in normal aged brain (23). Normal ataxin-3 is also readily recruited into inclusions...
formed by polyQ proteins in cell and animal models (15, 24–
27). The presence of UIMs in ataxin-3 raises the intriguing possibility that ataxin-3 is normally a ubiquitin-binding protein and that its colocalization to ubiquitin-conjugated protein inclusions is mediated by this property.

Using in vitro and cell-based methodologies, we now establish that ataxin-3 is indeed a poly-ubiquitin-binding protein, with binding mediated by UIMs situated in the polyQ tract. Our results, which confirm and extend recently published studies by others (28, 29), suggest that ataxin-3 differs from some UIM-containing proteins in that it binds poly-ubiquitin chains but not mono-ubiquitin. Our studies further reveal that binding kinetics are markedly influenced by surrounding protein context.

**Materials and Methods**

**Plasmid Constructs**—The ataxin-3 expression constructs pcDNA3-myc-Atx-3(Q28) and pcDNA3-myc-Atx-3(Q84) were described previously (26). Plasmid pcDNA3-myc-Atx-3(Q64) was generated by reverse transcription-PCR amplification of the 3′ fragment of ataxin-3 cDNA from patient lymphoblastoid cells and subcloning the sequence-verified product into pcDNA3-myc-Atx-3(Q64) (15). pcDNA3-myc-Atx-3(Q84) was generated through splice overlap extension PCR using primers that eliminated the CAG repeat. pcDNA3-myc-Atx-3(Q50) was generated during a PCR reduction to an intermediate length repeat using primers: pHis8-Atx-3(Q22)-S→A; pHis8-Atx-3-Nterm were kindly provided by Dr. Claudio Joazeiro (Genomics Research Institute, the Novartis Foundation, San Diego, CA). pGEX-4T-I-3(Q300), pGEX-4T-I-Atx-3(Q84), pGEX-4T-I-Atx-3(Q50), pGEX-4T-I-Atx-3(Q47), pGEX-4T-I-Atx-3(Q64), and pGEX-4T-I-Atx-3(Q64) were constructed by digesting the corresponding pcDNA3-Atx-3 expression plasmids with BamHI and cloning the released ataxin-3 fragment into pGEX-4T-I (Amersham Biosciences). pcDNA3-myc-Atx-3(Q22-C2WT) and pcDNA3-myc-Atx-3(Q22-C2S→A) were generated by PCR amplification using pcDNA3-myc-Atx-3(Q22)-WT or pcDNA3-myc-Atx-3(Q22)-S→A as the template DNA, respectively; the PCR product was then digested with BamHI/NotI and subcloned into pcDNA3-myc plasmid. pHis8-Atx-3(Q22-C2WT) and pHis8-Atx-3(Q22-C2S→A) were constructed by digesting the corresponding pcDNA3-myc-Atx-3 plasmids with BamHI/NotI and subcloning the released ataxin-3 cDNA into pHis8 expression vector (kindly provided by Dr. Claudio Joazeiro, Novartis). All plasmids generated by us were verified by restriction enzyme digest and sequence analyses.

**Cell Transfection and Co-immunoprecipitation**—Methods for cell culture, transfection, immunofluorescence, co-immunoprecipitation, and Western blot were described previously (15, 21, 24, 26). Stably transfected, doxycycline-inducible PC6–3 cell lines were generated as described previously (30). The clones PC6–3–ataxin-3(Q28)#23, PC6–3–ataxin-3(Q84)#18, and PC6–3–ataxin-3(Q108)#9 were chosen because they were highly inducible and exhibited robust ataxin-3 expression.

For the ataxin-3 ubiquitinated protein co-immunoprecipitation experiments stably transfected, doxycycline-inducible PC6-3 cell lines were cultured with doxycycline for 24 h and then treated with 10 μM lactacystin overnight. The cells were solubilized with nondenaturing lysis buffer (0.5% Nonidet P-40, 150 mM NaCl, 7.4, 0.2 mM HEPES, 1 mM EDTA) supplemented with 20 μM phenylmethylsulfonyl fluoride, 200 μM leupeptin, and 5 μM pepstatin A. Cell lysates were precipitated with monoclonal anti-ubiquitin antibody. The resultant immunoprecipitates were boiled 5 min, and analyzed by Western blotting with monoclonal anti-ubiquitin antibody.

**Protein Purification**—Glutathione S-transferase (GST)-tagged and histidine (His)-tagged constructs were transformed into BL21 competent cells. For His-tagged constructs and GST-tagged constructs single histidine (His)-tagged constructs were transformed into BL21 competent cells. For His-tagged constructs and GST-tagged constructs single histidine (His)-tagged constructs were transformed into BL21 competent cells. For His-tagged constructs and GST-tagged constructs single histidine (His)-tagged constructs were transformed into BL21 competent cells. For His-tagged constructs and GST-tagged constructs single histidine (His)-tagged constructs were transformed into BL21 competent cells. For His-tagged constructs and GST-tagged constructs single histidine (His)-tagged constructs were transformed into BL21 competent cells. For His-tagged constructs and GST-tagged constructs single histidine (His)-tagged constructs were transformed into BL21 competent cells.
that recognizes mono-ubiquitin and poly-ubiquitin. As shown in Fig. 2, both normal and expanded ataxin-3 co-precipitated ubiquitinated proteins, which electrophoresed on gels as a high molecular weight smear. Reprobing blots with an antibody recognizing mono-ubiquitin and poly-ubiquitin reveals a similar high molecular weight smear (not shown), indicating that normal and expanded ataxin-3 bind poly-ubiquitinated proteins either directly or indirectly.

The presence of three predicted UIMs in ataxin-3 suggested to us that ataxin-3 co-precipitates ubiquitinated proteins by directly binding to ubiquitin conjugates. To test this we performed in vitro binding assays between recombinant ataxin-3 (Fig. 1C) and mono-ubiquitin (ub), K48-linked di-ubiquitin (ub2), or K48-linked tetra-ubiquitin (ub4). Bacterially expressed GST-ataxin-3 fusion proteins were purified (Fig. 3A), then used in glutathione pull-down experiments to assess ataxin-3 binding to ub, ub2, and ub4. For initial experiments we used the originally published ataxin-3 isoform (9) that contains only the first two UIMs, because this isoform has been most extensively studied.

As shown in Fig. 3B, K48-linked ub4, but not ub or ub2, co-precipitated with GST-ataxin-3, indicating direct binding of ub4 to ataxin-3. Even with the shortest possible washes during co-precipitation (~4 min), we were unable to detect ataxin-3 binding to ub or ub2 (not shown). Accordingly, all further precipitation assays focused on ub4 binding.

In Vitro Analysis of the Role of PolyQ and UIMs in Ubiquitin Binding by Ataxin-3—Additional glutathione pull-down experiments with recombinant ataxin-3 lacking a polyQ domain (QHQ only) demonstrated that a polyQ tract was not necessary for ub4 binding by ataxin-3 (Fig. 3C). Moreover, ataxin-3 that contained expanded polyQ domains of 50 or 84 repeats still co-precipitated Ub4 (Fig. 3C), indicating that the pathogenic form of ataxin-3 retains the capacity to bind ubiquitin. The amount of ub4 co-precipitated by expanded ataxin-3, however, appeared to be reduced. This suggested that polyQ expansion leads to quantitative differences in ubiquitin binding, a possibility that is directly tested later in biosensor binding studies.

To determine whether ubiquitin binding by ataxin-3 is mediated by the UIMs, we performed further binding studies with a His-tagged, alternatively spliced form of ataxin-3 containing a normal polyQ domain and all three UIMs. His-ataxin-3 proteins were purified from bacterial lysates by nickel affinity chromatography, then incubated with ub, ub2, or ub4 followed by a second round of affinity purification. As with GST-ataxin-3, His-ataxin-3 did not bind (i.e. co-precipitate) ub or ub2 (data not shown) but did bind ub4. Accordingly, only data obtained with ub4 are displayed in Fig. 4. Full-length ataxin-3 co-precipitated ub4 as did the C-terminal half of ataxin-3 containing all three UIMs but lacking the polyQ domain. In contrast, the N-terminal half of ataxin-3, which contains the conserved polyQ domain but no UIMs, did not co-precipitate ub4. Thus, ub4 binding localizes to the UIM-containing, C-terminal half of ataxin-3.

To test the importance of UIMs in ubiquitin binding by ataxin-3, we performed similar studies with His-ataxin-3 in which the critical serine residue in each UIM was converted to alanine. This alanine-substituted ataxin-3 failed to bind ub4 (Fig. 4). Moreover, a C-terminal ataxin-3 fragment containing the same three alanine-substituted UIMs showed very little, if any, ub4 binding in this assay. Thus, one or more UIMs is essential for ubiquitin binding by ataxin-3.

Surface Plasmon Resonance Analysis of Ubiquitin-ataxin-3 Interactions—The preceding co-precipitation studies provided us with a qualitative assessment of ubiquitin binding by ataxin-3 but did not permit rigorous analyses of binding properties. Even relatively subtle changes in ubiquitin binding kinetics or affinity could have significant implications for disease pathogenesis in SCA3/MJD. Because expanded ataxin-3 appeared to bind relatively less ub4 in our glutathione pull-down assays (Fig. 3C), we next sought to determine whether expansion of polyQ does in fact alter ubiquitin binding by ataxin-3.

We used the BIAcore 3000 instrument to monitor binding between (poly)ubiquitin and ataxin-3 proteins, manifested as
changes in surface plasmon resonance. In our case, ubiquitin conjugates were immobilized on the CM5 sensor chip and recombinant ataxin-3 represented the analyte, which was injected across the surface of the chip at various concentrations (0–1600 nM). An analysis of changes in surface plasmon resonance that occur when ataxin-3 is injected across the chip provides a measure of the (poly)ubiquitin binding kinetics.

Ub2 and ub4 were attached to different quadrants of the same sensor chip, permitting simultaneous acquisition of binding data for both ubiquitin conjugates (ub could not be attached successfully to the sensor chip). In our initial BIAcore studies ataxin-3 did not bind ub2 (data not shown), consistent with our earlier co-precipitation results showing that ataxin-3 does not bind ub or ub2 but only ub4. Thus, all further BIAcore studies focused on binding to ub4, although in all experiments we also simultaneously collected negative binding data with ub2.

Ataxin-3 reproducibly showed high affinity binding to ub4 (Fig. 5). When ataxin-3 was washed off the chip the slow change in surface plasmon resonance indicated a relatively slow dissociation. The calculated equilibrium dissociation constant \( K_D \) was 2.0 \( \times 10^{-7} \) M (Table I). The N terminus of ataxin-3 did not bind ub4, confirming that the domain responsible for binding poly-ubiquitin resides in the C-terminal half of ataxin-3. Ataxin-3 with alanine-substituted UIMs also showed no detectable binding across a broad concentration range. Thus, BIAcore analysis confirmed that one or more UIMs are essential for ubiquitin binding by ataxin-3.

Because all three UIMs map to the C terminus of ataxin-3, we next tested whether the C terminus alone could recapitulate all aspects of ubiquitin binding by ataxin-3. As shown in Fig. 6 and Table I, the C terminus still bound ub4 with high affinity. The kinetics of binding, however, differed from that observed with full-length ataxin-3. The on and off rates for ub4 binding were much faster for the C terminus than for the full-length protein. The off rate in particular was more than 9-fold faster for the C terminus, and the on rate was ~4-fold faster. The calculated \( K_D \) for ubiquitin binding by the C terminus remained in the submicromolar range (Table I).

Having established a quantitative ubiquitin binding assay, we then tested whether polyQ expansion alters binding properties. For these experiments, we used a GST fusion of the ataxin-3 splice variant that contains two UIMs, since this isoform has been used most extensively in studies of polyQ protein aggregation (15, 21, 24, 26, 36, 37). Ataxin-3 containing either no polyQ tract (QHQ), a normal tract (Q28), or a modestly expanded tract (Q64) were all tested for ub4 binding. An expanded repeat length of 64 glutamine residues is well within the disease repeat range (55–84), yet not so large as to confound our ability to express and purify recombinant protein. As shown in Fig. 7 all three ataxin-3 proteins bound to ub4 with nearly identical binding curves. For all three the calculated binding kinetics and affinities were similar (Table I). The fact that GST-ataxin-3 with two UIMs had similar binding properties to His-ataxin-3 with three UIMs suggests that both major ataxin-3 splice isoforms bind poly-ubiquitin with similar affinity. The data also suggest that the third UIM is dispensable for ubiquitin binding by ataxin-3, consistent with recently published results by Donaldson et al. (28).
Poly-ubiquitin/Ataxin-3 Interactions

In this study we have established that the SCA3/MJD protein ataxin-3 is a poly-ubiquitin binding protein. Our results, which confirm and extend recent studies published by others (28, 29), define an intrinsic property of this disease protein that should lead to a better understanding of its primary cellular functions. Moreover, because the ubiquitin-proteasomal pathway has already been implicated in polyQ disease pathogenesis (7), the findings described here raise the possibility of important links between normal ataxin-3 biology and mechanisms of neurodegeneration in SCA3/MJD and other polyQ diseases.

Many UIM-containing proteins participate in nonproteasomal ubiquitin pathways, often by interacting with mono-ubiquitinated substrates. In the assays we employed, however, ataxin-3 showed no capacity to bind mono-ubiquitin or di-ubiquitin. In solution, UIM peptides bind mono-ubiquitin with relatively low affinity, with dissociation constants approaching the millimolar range (38). It is, therefore, possible that low affinity binding to mono- or di-ubiquitin would have been missed in our pull-down assays, as they require extensive washes of the resin-bound complexes. The absence of real-time binding to Ub2 in our surface plasmon resonance studies argues against this, although the relatively low concentrations of ataxin-3 used in these studies (1.6 μM being the highest) do not allow us to exclude this possibility entirely. Based on our data, we favor the view that ataxin-3 exclusively binds poly-ubiquitinated substrates.

UIMs 1 and 2 of ataxin-3 flank one another in the protein, reminiscent of many other proteins with two UIMs (e.g. several epsins). In such proteins the UIMs may cooperate to present the surface that mediates ubiquitin binding. Further surface plasmon resonance analyses with ataxin-3 engineered to contain a single functional UIM should provide insight into the molecular determinants that underlie the specificity for polyubiquitin. Another factor that may regulate ubiquitin binding is intermolecular interaction either between ataxin-3 mono- or between ataxin-3 and other cellular proteins. In immunoprecipitation and yeast two-hybrid studies ataxin-3 interacts with itself and with other proteins. Moreover, ataxin-3 sediments broadly through sucrose density gradients, presumably partly within large protein complexes of unknown composition (15). If ataxin-3 forms homodimers in cells, the topography of the UIMs presented by such a complex could modulate ubiquitin binding specificity. Similarly, in larger protein complexes ataxin-3-interacting proteins might alter the accessibility of the UIMs, further modulating ubiquitin binding by ataxin-3. Clearly, a full understanding of ataxin-3 interactions with ubiquitin requires more information about the quaternary structures adopted by ataxin-3 in cells.

All our in vitro binding studies were performed with K48-linked poly-ubiquitin, the most common ubiquitin-ubiquitin linkage in cells. A critical issue will be to determine which other forms of poly-ubiquitin, if any, can bind ataxin-3 and, equally important, what kinds of ubiquitin-ataxin-3 interactions actually take place in the cellular milieu. The fact that only ub4 binds to ataxin-3 in vitro does not rule out the possibility of physiologically significant interactions with shorter poly-ubiquitin chains or even mono-ubiquitinated proteins in living cells. Further studies employing techniques to assess ataxin-3/ubiquitin interactions in intact cells will be needed to address this issue.

The delivery of ubiquitinated substrates to proteasomes typically requires a minimum chain length of four ubiquitin molecules linked via K48 (39). The tight association of ataxin-3 with K48-linked poly-ubiquitin suggests that it participates in some manner in the ubiquitin- and proteasome-dependent degradation pathway. As we were writing this study, Madura and co-workers (29) reported that ataxin-3 binds to the proteasome directly, implying that ataxin-3 modulates ubiquitin-dependent proteasomal degradation through its ubiquitin binding properties. Although an interpretation of this result consistent with the model of Madura and co-workers is that excess ataxin-3 inhibits the delivery of ubiquitinated substrates to the proteasome, we have not detected proteasome impairment in ataxin-3-overexpressing cells that also express a reporter protein for proteasomal degradation. Based on a bioinformatics approach, Hofmann and co-workers (40) recently suggested another possible ubiquitin-linked function for ataxin-3; that the highly conserved Josephin domain of ataxin-3 possesses the predicted catalytic triad of amino acids found in ubiquitin-specific proteases. Indeed, since the completion of the current study, experimental evidence supporting this prediction has been reported (41). Studies in cells expressing mutated versions of ataxin-3 (i.e. lacking either functional UIMs or the catalytic triad) will be needed to define the precise role or roles ataxin-3 plays in normal ubiquitin pathways.

What are the physiological consequences of ubiquitin bind-

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**DISCUSSION**

FIG. 5. Surface plasmon resonance analysis of ataxin-3 binding to tetra-ubiquitin. Shown are changes over time in surface plasmon resonance, indicating binding between ataxin-3 and ub4. Three His-tagged ataxin-3 analytes, wild type (WT), UIM mutated (S→A), and N-terminal domain (Nterm), were individually injected at 1600 nM across the BIACore sensor chip to which ub4 had been immobilized. The blue arrow denotes the time of analyte injection, and the green arrow denotes the shift to buffer wash. Only full-length ataxin-3 led to changes in surface plasmon resonance. RU, resonance units. Resp. Diff., response difference.

**Table 1**

| Analyte         | $k_a$ | $k_d$ | $K_D$ |
|-----------------|-------|-------|-------|
|                 | $M^{-1}s^{-1} \times 10^d$ | $s^{-1} \times 10^d$ | $M \times 10^{-7}$ |
| His-axt-3-Q22-WT | 1.92 ± 0.06 | 0.39 ± 0.03 | 2.02 ± 0.11 |
| His-axt-3-Q22C-WT | 7.74 ± 0.16 | 3.67 ± 1.24 | 4.78 ± 1.73 |
| GST-axt-3-QHQ    | 1.80 ± 0.07 | 0.79 ± 0.04 | 4.40 ± 0.42 |
| GST-axt-3-Q28    | 3.91 ± 0.02 | 0.99 ± 0.01 | 2.57 ± 0.04 |
| GST-axt-3-Q64    | 1.69 ± 0.32 | 0.93 ± 0.08 | 5.55 ± 0.49 |

2 Y. Chai, N. Bonini, and H. Paulson, unpublished observations.

3 H. Paulson and S. S. Berke, unpublished observations.
ing by ataxin-3? Its ability to bind poly-ubiquitin suggests a mechanism by which ataxin-3 is recruited to ubiquitin-positive inclusions. More than perhaps all other polyglutamine proteins, ataxin-3 is readily recruited to polyglutamine inclusions in various model systems and is sequestered in Marinesco bodies, the ubiquitin-rich neuronal inclusions found in normal aged brain (23). Donaldson et al. (28) recently showed that ataxin-3 lacking functional UIMs is no longer efficiently recruited to inclusions in transfected cells, supporting the view that ubiquitin binding facilitates ataxin-3 recruitment to inclusions. This then begs the question, What function does ataxin-3 serve in inclusions? As a poly-ubiquitin-binding protein, does ataxin-3 aid the cell in handling aggregated protein within inclusions? And does the ubiquitin binding property of ataxin-3 modulate its own tendency to form insoluble inclusions? Answering these questions will require further studies, including analyzing polyQ protein aggregation in cells where ataxin-3 expression is suppressed and expressing UIM-mutated forms of ataxin-3 in cellular and animal models to assess their effect on polyglutamine aggregation and toxicity.

An issue relevant to SCA3/MJD pathogenesis is whether polyQ expansion in ataxin-3 alters ubiquitin binding in intact cells. Although our in vitro studies did not reveal a major polyQ length-dependent change in ubiquitin binding, the behavior of the purified proteins may differ substantially from that of the same proteins in a living cell. Expanded polyQ, for example, could alter ataxin-3 interactions with other proteins, which in turn would affect its ability to bind poly-ubiquitin. Even subtle changes might over the decades-long course of disease contribute to pathogenesis.

Finally, our studies highlight the importance of protein context in polyQ disease. It is increasingly clear that the details of pathogenesis in any polyQ disorder are partly determined by the full sequence of the disease protein and its biological functions (8). The fact that full-length ataxin-3 and the C-terminal fragment of ataxin-3 differ markedly in ubiquitin binding kinetics suggests that N-terminal regions of the protein influence ubiquitin binding. Indeed, the N-terminal josephin domain of ataxin-3 is the most highly conserved part of the protein and is itself linked to ubiquitin pathways. This domain mediates ataxin-3 interactions with HHR23A/B (42), the human homologs of Rad23, a yeast protein that may negatively regulate ubiquitination (43, 44), and the josephin domain may form a ubiquitin-specific protease (40, 41). The altered ubiquitin binding kinetics we observed could reflect differences in the oligomeric state of ataxin-3, as discussed above. Alternatively, the N terminus of ataxin-3 may constrain the allowable structures adopted by UIMs in ataxin-3. Further binding analyses of mutant forms of ataxin-3 coupled with experiments to assess the sizes of protein complexes should provide insight into this issue.

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