Soluble Androgen Receptor Oligomers Underlie Pathology in a Mouse Model of Spinobulbar Muscular Atrophy

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In polyglutamine diseases such as X-linked spinobulbar muscular atrophy (SBMA), it is unknown whether the toxic form of the protein is an insoluble or soluble aggregate or a monomer. We have addressed this question by studying a full-length androgen receptor (AR) mouse model of SBMA. We used biochemistry and atomic force microscopy to immunopurify oligomers soluble after ultracentrifugation that are comprised of a single ~50-kDa N-terminal polyglutamine-containing AR fragment. AR oligomers appeared several weeks prior to symptom onset, were distinct and temporally dissociated from intranuclear inclusions, and disappeared rapidly after castration, which halts disease. This is the first demonstration of soluble AR oligomers in vivo and suggests that they underlie neurodegeneration in SBMA.

Polyglutamine diseases such as X-linked spinobulbar muscular atrophy (SBMA) derive from CAG codon repeats that exceed a crucial length. This creates elongated polyglutamine tracts in affected proteins and progressive neurologic dysfunction and neurodegeneration (1). SBMA results from expanded glutamine repeats in the N terminus of the androgen receptor (AR) protein (2, 3). It is characterized by slowly progressive lower motor neuron degeneration and mild sensory neuropathy that predominates in males (4, 5) due to exposure to androgens (6, 7). Large intracellular inclusions containing AR are observed throughout the body and may be localized in the cytoplasm or nucleus, whereas large intranuclear inclusions accumulate preferentially in motor neurons (8–10). Pathologically expanded polyglutamine protein fragments are clearly prone to misfolding and aggregation in vitro that respects the tight length-dependent disease threshold seen in vivo (11, 12). Thus, protein misfolding and/or aggregation are very likely to underlie pathogenesis of SBMA and other polyglutamine diseases. In many experimental systems, however, large intracellular inclusions can be dissociated from neuronal toxicity, indicating that these structures are probably not the primary pathologic species (13–19).

If large inclusions are not pathogenic, what then is the toxic species? Most cellular and animal studies indicate that expanded proteins either exist as monomers or accumulate as large macromolecular complexes that are unable to enter an SDS-polyacrylamide gel (20–23). However, in vitro studies indicate clearly that polyglutamine proteins, like other amyloidogenic proteins, can form small, ordered oligomers (11, 24–27), and the existence of toxic, submicroscopic aggregates or oligomers has been invoked as a cause of polyglutamine toxicity in vivo (27, 28). The operative definition of such species has varied. We define oligomers here as submacromolecular structures (i.e. soluble after high speed centrifugation) comprised of ordered polyglutamine aggregates. As previously proposed by Taylor et al. (28), we contrast protein oligomers, which are defined primarily via biochemistry, from intracellular inclusions, which are defined via histopathology and are difficult to quantify via biochemistry.

An extensive literature supports the concept that soluble protein oligomers have cellular toxicity in vitro. Recent work has also suggested that soluble A-β oligomers are an important source of toxicity in vivo (29). It has also been suggested that oligomers might mediate neurotoxicity in diseases such as Parkinson disease and amyotrophic lateral sclerosis (30). In contrast, the production of large macromolecular inclusions is likely an end-stage consequence of the cell processing toxic proteins (30), although these structures may also have negative physiologic consequences (31, 32). To date, there has been no conclusive evidence for the existence of soluble polyglutamine protein oligomers in patients or animal models, nor any evidence that these species actually underlie toxicity in vivo. Green and colleagues (33) reported large formic acid non-dissociable “oligomers” in Huntington disease brain but did not clearly determine the nature of their protein constituents or physical properties. Establishing that oligomers are a key pathologic species in vivo would advance our basic understanding of disease mechanisms and help in developing new therapies and in monitoring disease onset and progression. In this study, we have used a full-length AR mouse model of SBMA to identify...
and characterize oligomers made up of N-terminal AR fragments and to determine their abundance at different stages of disease onset, progression, and regression.

**EXPERIMENTAL PROCEDURES**

**Mouse Models**—Mice used in this study have been characterized previously in a different background strain (7). These transgenic animals harbor a full-length human AR gene containing 112 CAG repeats (AR112) driven by the PrP promoter or wild-type human AR with 24 CAG repeats (AR24). Founder male mice were backcrossed with C57B/6 females (Jackson Laboratory, Bar Harbor, ME) to more than seven generations. Genotyping was performed from tail DNA by PCR of the human AR as described previously (7). Sequencing PCR products from a seventh generation of mouse genomic DNA of an AR 112 male indicated 113 CAG repeats. Mice were maintained in a barrier animal facility at the University of California San Francisco or at Thomas Jefferson University, housed five per cage with food and water available ad libitum, and kept on a 12-h light/dark cycle. All studies and procedures were done under the approval of the Institutional Animal Care and Use Committees in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Castration**—Castration was performed on AR112 males as described previously (7). Animals were euthanized, and tissues were harvested for pathology and biochemistry 3 weeks after surgery.

**Rotarod Performance**—We performed rotarod evaluations, blinded to genotype, for wild-type and AR112 males on a rotarod apparatus (Columbus Instruments, Columbus, OH) as described previously (7). Briefly, we carried out four rotarod tests per session, allowing at least 15 min of rest for the mice between each test. The rotarod accelerated from 4 to 40 rpm over 15 s and was maintained at 40 rpm thereafter. The lowest value from all four tests was discarded. The remaining three values were averaged, and the data were analyzed by Student’s t test, assuming equal variance between the two groups.

**Tissue Dissection and Homogenization**—Unfixed brains were removed whole and cut in a midsagittal section. One-half of the brain tissue was homogenized either by brief sonication for 5 min at 4°C for Triton or RIPA buffer and centrifuged at 15,000 × g for 30 min at 4°C (Triton buffer) or 15,000 × g for 30 min at 4°C (RIPA buffer). 2 μl of rabbit anti-AR N-terminal specific antibody (N-20, Santa Cruz Biotechnology) was conjugated to a 20-μl bead volume of goat anti-rabbit IgG beads (Sigma) in washing buffer (10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 1% Triton X-100, and protease inhibitors) or RIPA buffer. 200 μl of protein supernatant extracted with the above high salt TBS-sucrose-Triton buffer (diluted 5-fold with water) or RIPA buffer was added with AR N-20-rabbit IgG beads overnight at 4°C. Beads were washed thoroughly with washing buffer or RIPA buffer and divided into two equivalent aliquots. One was boiled for 5 min in 1% SDS sample buffer, and the other was mixed with 100 μl of 100% formic acid, incubated at 37°C with constant shaking at 250 rpm for 1 h, and dried by a centrifuge vacuum. Dried beads were resuspended in 1% SDS sample buffer with 5% β-mercaptoethanol and 0.004% bromphenol blue, with the pH adjusted with 5 μl of saturated Tris base (pH 11.25), and boiled at 100°C for 5 min. Samples were run immediately on an SDS-PAGE gel followed by Western blot analyses. Equal amounts of lysate (12 μl) were resolved by 4–15% gradient SDS-PAGE (Bio-Rad Laboratories) and transferred to polyvinylidene difluoride membrane (pore size 0.45 μm) using a semidry transfer apparatus. 5% nonfat milk was used to block the membrane. Blots were probed with polyclonal anti-AR N-20 (1:2000 dilution), washed with TBS and 0.5% Tween 20, and then incubated with horseradish peroxidase-conjugated rabbit True Blot (1:5000 dilution, eBioscience) that does not recognize denatured rabbit IgG heavy or light chain. Horseradish peroxidase signal was detected by ECL-Plus (Amersham Biosciences). Stripping buffer (62.5 mM Tris-HCl, pH 6.7, 2% SDS, and 100 mM β-mercaptoethanol) was used for repeat blots, incubated at 55°C for 1 h with occasional shaking, and reprobed with monoclonal anti-expanded polyglutamine (1C2, 1:2500 dilution, Chemicon International) or monoclonal anti-AR amino acid 299–315 antibody (AR 441, 1:500 dilution, Santa Cruz Biotechnology).

**Immunofluorescence**—Brain and spinal cord embedded in OCT were cut on a cryostat (Leica 3000) at −20°C. 12-μm sagittal brain and axial spinal cord sections were air-dried for 20 min. Dried sections were fixed with 4% paraformaldehyde for 10 min at 4°C, washed with 0.05% Tween 20 in phosphate-buffered saline, and then incubated with 5% goat serum and 0.1% Triton X-100 in phosphate-buffered saline for 30 min at room temperature followed by anti-AR N-20 (1:1000) overnight incubation at 4°C and washing with 0.05% Tween 20 in phosphate-buffered saline. Goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (1:4000; Molecular Probes) was applied to the sections for 2 h at room temperature in the dark. The sections were washed for 1 h and counterstained with 4',6-
AR Oligomers Underlie Pathology in a Mouse Model of SBMA

FIGURE 1. Intermediate molecular weight aggregates form only in AR112 male mice. A and B, frontal cortical sections of 6.5-month-old AR112 mice stained with AR N-terminal specific antibody (N-20). Male AR112 mice exhibit prominent intranuclear inclusions (A), whereas female mice do not (B). C, Western blots of brain extracts probed with N-20 demonstrate intermediate molecular weight AR aggregates (~250–450 kDa) only in AR112 male mice. Wild-type male (wtM) and wild-type female (wtF) mice, unexpanded AR24 transgensics, and AR112 females do not show such species. D, ultracentrifugation does not affect the presence of aggregated AR species. Brain specimens from AR112 mice of various ages were compared using ultracentrifugation at 150,000 × g (U) or standard centrifugation at 15,000 × g (S). The vertical bar illustrates the extent of aggregates. Dark arrow, full-length AR12; gray arrow: full-length wild-type AR. NS, nonspecific bands serve as a loading control.

RESULTS

Formation of Intermediate MW Aggregates in AR112 Mice—We previously described the generation of a mouse model of SBMA that is based on neuronal expression from the PrP promoter of full-length human AR with 112 glutamine repeats (AR112) along with an identical cDNA with 24 glutamine repeats (AR24) (7). In the C57B/6 background, AR112 males exhibit hormone- and age-dependent weakness beginning at ~10 weeks of age. Large neuronal intranuclear inclusions form at later time points throughout the central nervous system (7) (Fig. 1, A and B). We tested for soluble protein aggregates initially by homogenizing brain samples from 6-month-old animals in SDS buffer with brief sonication and clarifying the lysate via centrifugation at 15,000 × g. As described previously (7), after Western blot using AR N-terminal antibody (N-20, Santa Cruz Biotechnology), we observed AR112 monomer (~130 kDa) and an intermediate molecular weight smear consistent with aggregated protein running at ~250–450 kDa (Fig. 1C). Similar species were observed in spinal cord extracts, although overall levels of AR detected were much less (Supplemental Fig. 1). The aggregated species were not present in non-transgenic or female AR112 littermates or in male AR24 transgenics (Fig. 1C). Ultracentrifugation of tissue preparations (150,000 × g) had no effect on the prevalence of the intermediate MW species (Fig. 1D), indicating their high solubility. We excluded the possibility that sonication or SDS-extraction was somehow liberating the intermediate MW species from large aggregates by extracting brain samples without sonication in a non-ionic detergent (1% Triton X-100) not known to dissociate polyglutamine aggregates. We then carried out immunoprecipitation with N-20 antibody followed by Western blot. The intermediate MW species reactive with an AR N-terminal antibody remained and were found exclusively in symptomatic male AR112 mice (Fig. 2A).

diamidino-2-phenylindole (50 ng/ml) for 30 s, washed, and mounted with ProLong Gold anti-fade reagent (Molecular Probes). Fluorescence images were collected with a Zeiss Axiovert S100 fluorescence microscope and Zeiss Pascal laser confocal microscope.

Affinity Purification and AFM—Transgenic animals and their littermates were sacrificed at age 75 and 135 days. Whole brains were immediately removed and Dounce-homogenized in 1 ml of hypotonic buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 10 mM EDTA, 1 mM dithiothreitol, 0.4% IGEPAL (Sigma), plus protease inhibitors) per 500 mg of wet tissue. They were incubated for 15 min on ice and centrifuged at 850 × g at 4 °C, and the supernatant was discarded. The pellet was resuspended in hypertonic buffer (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, plus protease inhibitor mixture), incubated, and constantly mixed on ice for 30 min. After centrifugation at 15,000 × g for 30 min at 4 °C, the supernatant was diluted with Gentle Ag/Ab binding buffer (Pierce) plus protease inhibitors and N-20 covalently conjugated to protein G agarose beads. Samples were incubated overnight at 4 °C, washed with binding buffer four times, and eluted with Gentle Ag/Ab elution buffer (high salt buffer, pH 6.5, Pierce). Approximately 1 μg of eluted protein was immediately spotted on freshly cleaved mica, incubated for 2 min, washed twice with distilled water, and air-dried. The samples were imaged in air by a digital multimode Nanoscope III scanning probe microscope (Digital Images, Santa Barbara, CA). The atomic force microscopy (AFM) images were analyzed using WsxM 2.2 software from Nanotecnica Electronica.

Ex Vivo Fibrillation—Whole brain was homogenized in RIPA buffer with protease inhibitor mixture, incubated on ice for 20 min, and then centrifuged at 15,000 × g at 4 °C for 30 min. The supernatant was passed through a cellulose acetate filter and then eluted by 0.1M glycine-HCl buffer (pH 2–3) and then passed through a cellulose acetate filter and then eluted by 0.1M glycine-HCl buffer (pH 2–3). The beads were extensively washed with RIPA buffer and eluted by 0.1 M glycine-HCl buffer (pH 2–3) and then passed through a 0.45-μm cellulose acetate membrane filter by centrifugation at 5000 × g for 1 min. The pH was neutralized by adding 1 M Tris-HCl (pH 9.5) immediately after the elution. A 20-μl elution aliquot was added on the mica surface, washed twice with distilled water, and dried in air. As described previously (7), after Western blot using AR N-terminal antibody (N-20, Santa Cruz Biotechnology), we observed AR112 monomer (~130 kDa) and an intermediate molecular weight smear consistent with aggregated protein running at ~250–450 kDa (Fig. 1C).
Soluble Aggregates Consist of Formic Acid-dissociable N-terminal Fragments—In AR112 mice and SBMA patients, we have previously observed that neuronal intranuclear inclusions do not contain epitopes reactive with antibodies directed at the C terminus of AR, suggesting that they are comprised primarily of N-terminal fragments (7, 8, 34). Thus, we determined whether the intermediate MW species represented covalently modified AR protein or accumulations of dissociable monomer. We prepared brain extracts from 102-day-old male and female AR112 mice, which have no large inclusions, and immunoprecipitated AR using N-20 antibody. The beads were subsequently treated with or without 95% formic acid (FA) for 1 h, which has been demonstrated to dissociate SDS-insoluble protein aggregates that are stabilized by hydrogen bonds (33, 35, 36) but not to cleave isopeptide bonds (37). In the absence of FA treatment, we observed the intermediate MW aggregated AR species as described previously. With FA treatment, however, these species dissociated, and we observed a new ~50-kDa band consisting of a nonspecific band that serves as a loading control. A faint, nonspecific 50-kDa band that does not react with 1C2 is present in the AR112F sample, and a smaller band at ~40 kDa represents a nonspecific band that serves as a loading control. wtM, wild-type male mice; wtF, wild-type female mice. B, reprobing the blot with the 1C2 antibody, which reacts with non-aggregated expanded polyglutamine tracts, revealed the same ~50-kDa band observed in A that is released by formic acid treatment. C–J, atomic force microscopy was used to resolve material recovered by AR affinity purification and elution with high salt buffer. AR112 female mice (C and D) did not have evident oligomers, whereas samples from AR112 male mice (E and F) contained ordered oligomers ~4–15 nm in height that were arranged singly or in small proto-fibrillar arrays. A higher magnification picture (G and H) illustrates a small proto-fibril. I represents a height scan across the area pictured in several 5–10-μm² scan pictures, demonstrating the number of particles at each height. J represents a height scan across the protofibril pictured in G and H, indicating a height of ~7 nm. Images C, E, and G indicate amplitude images; images D, F, and H reflect actual heights of the samples. Note: The scale bars represent exact horizontal distance, but horizontal dimensions of detected particles cannot be accurately reflected by AFM; only the height scan is quantitative.
the full-length and the N-terminal fragments but not with unexpanded AR, as expected (Fig. 2B). We did not observe the ~50-kDa fragment in the absence of FA treatment, implying that this peptide does not constitute a significant amount of the cellular protein, is rapidly degraded, or rapidly aggregates. Our data suggest that the aggregates consist of accretions of N-terminal fragments rather than covalently modified AR monomer.

We characterized the aggregated species further by using non-denaturing immunopurification coupled with AFM. Brain extracts were immunoprecipitated under non-denaturing conditions with N-20 antibody conjugated covalently to protein G agarose beads. The precipitate was eluted with high salt buffer and placed immediately on mica for imaging via AFM at nanometer resolution. Although wtAR (data not shown) and female AR112 mice exhibited no particles (Fig. 2, C and D), extractions from 4.5-month-old AR112 male mice contained small particles ~7 nm in height, consistent with N-terminal AR oligomers (Fig. 2, E–H). Additionally, we observed occasional short proto-fibrils consisting of linked oligomers (Fig. 2, E–H). These observations are consistent with other reports of polyglutamine fibrils identified in the insoluble material from mice expressing htt exon 1 (22) and oligomers and fibrils that form in vitro (25, 38). We further characterized the oligomers present on the mica using height distribution analysis on several 5–10-μm² scan pictures (Fig. 2, I and J). This demonstrated that most particles were under 10 nm in height.

Single Oligomers Purified from Brain Self-assemble into Proto-fibrils—Oligomers retain the capacity to self-associate into ordered structures, as opposed to amorphous aggregates, which do not. Thus, we tested the ability of the particles identified in SBMA mouse brain to form linear, proto-fibrillar structures. First, we created lysates from 75-day-old animals as described previously, using centrifugation at 15,000 g. Next, we passed this lysate through a 0.45-μM cellulose acetate filter to eliminate larger aggregates. We purified AR species from this...
AR Oligomers Underlie Pathology in a Mouse Model of SBMA

FIGURE 5. Castration rapidly eliminates oligomers. A, four AR112 mice were castrated and compared with age-matched controls after 3 weeks. Brain extracts were evaluated by Western blot with N-20 antibody. Castration markedly reduced the amount of oligomers. The vertical bar illustrates the extent of aggregates. Dark arrow, full-length AR112; gray arrow, full-length wild-type AR; NS, nonspecific bands serve as loading control. Cast, castration. B, quantification of oligomer band intensities shows that there is a significant difference between castrated and non-castrated groups (p < 0.01; error bar represents the S.E.). C, 1C2 blot of immunoprecipitated brain extracts before and after castration. Castration eliminated the presence of the 50-kDa band that is liberated by formic acid extraction. Dark arrow, full-length AR112; wtM, wild-type male. D, immunofluorescence of cortical and spinal cord neurons from two intact versus castrated animals. Castration reduced the intensity of AR nuclear localization and slightly reduced the size but not the incidence of nuclear inclusions. The large inset on spinal cord samples is the higher power image of the small inset on each picture and shows the continued presence of inclusions after castration.

lysate using immunoprecipitation with N-20 antibody. We eluted the immunoprecipitated material and again passed the sample through a 0.45-μm cellulose acetate filter. One portion of the elution was immediately put on mica and imaged, whereas another portion of the 0-h elution was immediately added SDS sample buffer, boiled, and kept for further Western blot analyses. A remaining portion of the sample was incubated for 48 h at 4°C to allow for self-association to occur. The 48-h sample was then applied to a mica chip. The 0- and 48-h samples were then resolved together by SDS-PAGE and Western blot with N-20 antibody. Western blot revealed an increase in the amount of intermediate molecular weight species at 48 h without a change in the amount of full-length AR monomer (Fig. 3A). Likewise, AFM of the 48-h samples revealed protofibril structures consisting of linear arrangements of oligomers that were not detected in the 0-h samples (Fig. 3B and Supplemental Fig. 4). Height scans indicated no change in the overall height of the specimens, consistent with ordered arrays of oligomers rather than globular structures (Fig. 3B, Supplemental Fig. 4).

Oligomers Precede Symptom Onset and Are Temporally Distinct from Inclusions—AR112 mice show early onset motor deficits and age-dependent pathology, including progressive neuronal intranuclear inclusions (7). This enabled us to test the relationship of oligomers to motor symptoms and intranuclear inclusions. In the C57B/6 background, motor symptoms begin in AR112 males between 67 and 81 days of age (Fig. 4A). To compare the onset of oligomers and intranuclear inclusions, we studied 15 brain specimens derived from three sets of male transgenic AR112 littermates of various ages, ranging from 50 to 435 days. We evaluated the characteristics of intranuclear inclusions by immunofluorescence (Fig. 4, B and C). We did not observe large intranuclear inclusions until ~150 days of age in brainstem neurons (Fig. 4B) or frontal cortical neurons (Fig. 4C), although microscopic foci within the nucleus (nuclear speckles) were visible by ~70 days (Fig. 4B). As expected, the overall prevalence of inclusions progressed steadily over the life of the animals (Fig. 4C). In contrast, oligomers were detected by Western blot as early as 52 days (Fig. 4D). They reached a peak between 100 and 180 days and were detectable up to 435 days but with decreased abundance (Fig. 4D). Thus, we detected oligomers prior to the onset of motor dysfunction and found them to be independent of large intranuclear inclusions. This is most consistent with their role as the principal toxic species in SBMA.

Castration Rapidly Eliminates Oligomers—If oligomers underlie pathogenesis, they should disappear rapidly with disease-modifying therapy. We tested this idea by exploiting the hormone dependence of neurodegeneration in SBMA. We (7) and others (6) have observed that castration of mouse models of SBMA will prevent or reverse polyglutamine-induced pathology. This would be predicted to rapidly eliminate the presence of a toxic species. First, we selected eight age-matched male AR112 mice and castrated four of them. After 3 weeks, we sacrificed the animals and evaluated their brains via biochemistry and immunofluorescence as described previously. Oligomers were reduced in the castrated animals to levels comparable with the AR112 females without a significant change in the levels of full-length AR (Fig. 5, A and B). We also tested whether castration would eliminate production of the ~50-kDa band associated with the oligomers that is visible upon formic acid extraction. Without castration, we observed strong production of the ~50-kDa band using 1C2 antibody; after castration, this band entirely disappeared (Fig. 5C). Evaluation of cortex and spinal cord specimens of castrated and intact animals by immunofluorescence revealed a mild reduction in diffuse nuclear staining.
and in the size and number of the nuclear inclusions, although they were still readily visible after castration (Fig. 5D). Oligomers comprised of an ~50-kDa N-terminal fragment of AR are thus tightly linked to pathogenesis and resolve rapidly after disease-modifying therapy, prior to the disappearance of nuclear inclusions. This further implies a proximal role for these species in causing toxicity in vivo.

DISCUSSION

Prior studies of mouse models or patients with polyglutamine disease have not provided clear evidence of soluble oligomers, and so it has not been possible to test their pathogenic role. Previous work by us (7) and others (3, 6) has detected AR aggregates on Western blots from brain specimens that are similar to those characterized extensively here. La Spada and colleagues (3) described AR species that migrate below the stacking gel in Western blots from YAC AR100 mice and postulated that these might represent microaggregates or oligomers. Likewise, Sobue and colleagues (6) observed intermediate molecular weight species in Western blots from spinal cords and muscles in an SBMA mouse model. Green and colleagues (33) have described formic acid-resistant aggregated material isolated from inducible PC-12 cells and cortex from Huntington disease patients, which may represent a covalently modified form of polyglutamine protein. However, no previous work has characterized the intermediate molecular weight aggregates in detail, contrasted them with nuclear inclusions, or tested their relationship to central nervous system dysfunction.

Here we have provided the first evidence that soluble polyglutamine protein oligomers occur in vivo, linking their appearance with symptom onset and their disappearance with symptom resolution. Oligomers are present exclusively in the brains of male AR112 mice and are extractable in ionic or non-ionic detergents. They are dissociable in formic acid and appear to be comprised primarily of an ~50-kDa N-terminal AR fragment. Our initial detection of oligomers (52 days) occurred approximately 2–3 weeks prior to symptom onset. Conversely, oligomers disappeared within 3 weeks after castration, a treatment known to prevent disease and ameliorate motor symptoms in symptomatic mice (6, 7). Thus, the oligomers meet the essential criteria of a primary toxic agent.

The existence of an ~50-kDa fragment that comprises the oligomers suggests that full-length AR in vivo is subjected to a distinct proteolytic cleavage event following activation by ligand. We hypothesize that this initiates the accumulation of polyglutamine-containing AR N-terminal fragments within the cell. These fragments probably form oligomers and short proto-fibrils. At sufficient intracellular density, the oligomers may be accumulated into intranuclear inclusions. Taylor et al. (28) have observed that non-ionic detergent-insoluble aggregates are present with or without inhibition of microtubule-mediated transport, which they suggested might serve to accumulate small microaggregates into inclusions. Our data are consistent with this model and demonstrate that oligomers clearly precede formation of the large inclusions. We cannot exclude that a monomeric AR fragment is the primary toxic species and that it subsequently forms oligomers. This seems highly unlikely, however, since in the absence of FA-mediated dissociation, we observed no evidence of this fragment. We have also observed that the prevalence of oligomers falls over time, whereas the number of large intranuclear inclusions increases. Given the slowly progressive nature of the pathology, it is possible that the large intranuclear inclusions also play a role in the pathological process. However, the simplest interpretation of our data is that AR N-terminal oligomers constitute a primary pathological species in vivo. This general observation is consistent with emerging reports of pathological studies for Alzheimer disease, Parkinson disease, and tauopathy (29, 39, 40). Now that the appearance of soluble polyglutamine protein oligomers has been clearly linked to the onset and resolution of the disease process in transgenic mice, further purification and characterization may allow a precise definition of how they are formed and their mechanism of toxicity. This, in turn, may provide new therapeutic targets.

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AR Oligomers Underlie Pathology in a Mouse Model of SBMA

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