A Drosophila Homolog of the Polyglutamine Disease Gene SCA2 Is a Dosage-Sensitive Regulator of Actin Filament Formation

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Manuscript received June 6, 2002
Accepted for publication September 12, 2002

ABSTRACT

Spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative disorder caused by the expansion of a CAG repeat encoding a polyglutamine tract in ataxin-2, the SCA2 gene product. The normal cellular function of ataxin-2 and the mechanism by which polyglutamine expansion of ataxin-2 causes neurodegeneration remain unknown. In this study we have used genetic and molecular approaches to investigate the function of a Drosophila homolog of the SCA2 gene (Datx2). Like human ataxin-2, Datx2 is found throughout development in a variety of tissue types and localizes to the cytoplasm. Mutations that reduce Datx2 activity or transgenic overexpression of Datx2 result in female sterility, aberrant sensory bristle morphology, loss or degeneration of tissues, and lethality. These phenotypes appear to result from actin filament formation defects occurring downstream of actin synthesis. Further studies demonstrate that Datx2 does not assemble with actin filaments, suggesting that the role of Datx2 in actin filament formation is indirect. These results indicate that Datx2 is a dosage-sensitive regulator of actin filament formation. Given that loss of cytoskeleton-dependent dendritic structure defines an early event in SCA2 pathogenesis, our findings suggest the possibility that dysregulation of actin cytoskeletal structure resulting from altered ataxin-2 activity is responsible for neurodegeneration in SCA2.

THE polyglutamine repeat diseases are a group of at least eight dominantly inherited disorders characterized by progressive degeneration of specific neuronal populations and a shared mutational mechanism involving expansion of a glutamine-encoding repeat in the corresponding genes (Cummings and Zoghbi 2001). Similar characteristics among the different polyglutamine repeat disorders have stimulated intensive investigation of a common pathogenic mechanism. This work has led to the finding that nuclear localization of long polyglutamine tracts can trigger neurodegeneration through a transcriptional interference mechanism (La Spada et al. 2001; Shimohata et al. 2001; Steffan et al. 2001). While transcriptional interference may account for pathology in some of the polyglutamine repeat disorders, increasing evidence indicates that unique nuclear and nonnuclear pathways are involved in the mechanisms of neurodegeneration in the different polyglutamine disorders (Huynh et al. 2000; La Spada et al. 2001; Steffan et al. 2001). An alternative model to explain pathology in the polyglutamine repeat disorders is that pathogenesis results from an alteration of the normal cellular function of the polyglutamine disease gene as a consequence of polyglutamine expansion. Although data support this hypothesis for several polyglutamine repeat disorders (Dragatsis et al. 2000; Restittuto et al. 2000), this model has not been broadly tested, in part because the normal cellular functions of most polyglutamine repeat disease genes remain unknown.

One polyglutamine repeat disorder that does not appear to involve a transcriptional interference mechanism of neurodegeneration is spinocerebellar ataxia type 2 (SCA2). SCA2 is characterized by progressive loss of coordination and imbalance resulting from dysfunction and degeneration of Purkinje cells in the cerebellum. The loss of cerebellar Purkinje cells is preceded by loss of the Purkinje cell dendritic Arbor (Huynh et al. 2000). The SCA2 gene product, ataxin-2, is a 140-kD cytoplasmic protein of unknown function that is found broadly in brain and other tissue types (Huynh et al. 1999). The cytoplasmic distribution of ataxin-2 is preserved in at least some SCA2 subjects and in a mouse model of this disorder, indicating that SCA2 pathology has a cytoplasmic origin (Huynh et al. 1999, 2000). Although the function of the SCA2 gene is unknown, ataxin-2 contains amino acid sequences that suggest it may bind to poly(A)-binding protein (PABP; Kozlov et al. 2001), and yeast two-hybrid studies indicate that ataxin-2 interacts with a cytoplasmic RNA binding protein (Shibata et al. 2000). Together, these observations suggest that ataxin-2 functions in RNA metabolism.

To understand the mechanism of SCA2 pathogenesis, we are using Drosophila as a model system to investigate the normal cellular function of a SCA2 homolog (Datx2). In this study, we show that the Datx2 gene encodes a protein that is highly conserved with ataxin-2 in two do-
mains, including a putative PABP interaction domain. Like its human counterpart, Datx2 encodes a cytoplasmic protein present throughout development in a variety of tissue types, including the nervous system. Mutations that reduce Datx2 activity or transgenic over-expression of Datx2 result in severe phenotypic consequences, including dysfunction, loss, or degeneration of mesoderm, nerve, and other tissue types. Loss of Datx2 function in the retina, sensory bristles, and female germline results in cellular and tissue morphological changes indicative of actin filament formation defects. Moreover, loss of function or overexpression of Datx2 in these mouse tissues results in alterations in the structural characteristics of actin filaments. These phenotypes do not arise from alterations in the cellular abundance of actin, and Datx2 does not appear to assemble with actin filaments. Our results, coupled with other work linking several ataxin-2 family members to RNA metabolism (Mangus et al. 1998; Shibata et al. 2000; Kozlov et al. 2001), indicate that Datx2 is a dosage-sensitive regulator of actin filament formation, possibly acting to control translation, stability, or localization of transcripts encoding mediators of actin polymerization. These findings may be relevant to the mechanism of neurodegeneration in SCA2, since loss of the actin-dependent dendritic arbor of cerebellar Purkinje cells is an early event in SCA2 pathogenesis (Huynh et al. 2000). Our results provide a foundation for a direct test of this hypothesis.

MATERIALS AND METHODS

Molecular genetics and fly strains: Genomic and cDNA sequences encoding the Drosophila ataxin-2 homolog were identified by searching the Berkeley Drosophila Genome Project (BDGP) database (Adams et al. 2000; Rubin et al. 2000) using a human ataxin-2 polypeptide query sequence (AAB19200). One of the Datx2 cDNA clones (GH02324) was fully sequenced and this sequence was compared to genomic DNA sequence to identify splice junctions in the Datx2 gene. The validity of the splicing arrangements predicted by the GH02324 cDNA clone was then verified by analyzing a larger collection of Datx2 cDNA clones (GH27029, SD08349, GH13857, and LD47794) by restriction mapping, sequencing, and PCR. TheDatx2 polypeptide sequence encoded by the GH27029 cDNA was aligned to human ataxin-2 (AAB19200), human ataxin-2 related protein (NP_009176), Caenorhabditis elegans ATX-2 (D2045.1), Saccharomyces cerevisiae Pbp1p (NP_016194), and Arabidopsis thaliana polypeptide sequences (F1511.27 and MDC16.14) using the ClustalW algorithm. The developmental expression profile of Datx2 was determined using the Rapid-Scan semiquantitative PCR system (Origene Technologies, Rockville, MD) following conditions suggested by the manufacturer. The PCR primers used for amplification of Datx2 were 5′-GGGCTCTACAACAAG-3′ and 5′-CCGAGAATGTGC GGAAT-3′. These primers amplify sequences encoding the evolutionarily conserved ATXN-2 domain of Datx2. rp49 control primers were supplied with the cDNA panel. The insertion locations of the l(2)066H, Eph(3)3622, and Eph(3)3455 P elements were confirmed by recovery and sequencing of DNA flanking the P elements. The GH27029 cDNA clone was subcloned into the pUAST vector (Brand and Perrimon 1993) as a BglII-Xhol fragment and used to generate germline transgenic flies using standard procedures (Ashburner 1989). The Df(3R)Poa4 stock, which removes the Datx2 locus, was used in complementation and lethal phase analysis with the Datx2 mutants. A minimum of 100 progeny were scored for each of these experiments. Females with germline clones of the Datx2 mutations were generated using the FLP-DFS technique (Chou and Perrimon 1996). Datx2 mutations were generated by using a heat-shock-inducible FLP recombinase to drive mitotic recombination. With the exception of the Datx2 transgenic lines and the imprecise excision alleles of Datx2, all fly stocks described in this work were obtained from the Bloomington Stock Center or Berkeley Drosophila Genome Project.

Immunological methods: The Datx2 antiserum was commercially prepared by immunizing rabbits with a synthetic peptide corresponding to residues 151–165 in Datx2 (SDKNCARPDEKELE) conjugated to keyhole limpet hemocyanin (Research Genetics, Birmingham, AL). Actin and clav antisera were obtained from Chemicon (Temecula, CA) and the Developmental Studies Hybridoma Bank (Iowa City, IA), respectively. Antiserum to ADH was provided by Saverio Brogna. Western blot analyses were performed essentially as described by Tolar and Pallanck (1998). Wild-type embryos were collected, fixed, and stained according to standard procedures. Ovaries were dissected, fixed, and stained as described by Jackson and Berg (1999). Ventral ganglion tissues, eye discs, and thoraces were dissected, fixed, and stained in the same manner as ovaries. Fluorescence microscopy of stained ovaries was carried out using a Nikon MICROPHOT-FXA fluorescent microscope. Images were captured on 35-mm Kodak Ektachrome slide film and digitally scanned using a Polaroid Sprint Scan 35 digital slide scanner. Optical sections of stained tissues were obtained on a Bio-Rad (Richmond, CA) MRC600 confocal microscope and projected into a single plane in NIH Image.

Scanning electron microscopy: Adult flies were dehydrated by 15-min incubations in a graded ethanol series. The dehydrated flies were treated with hexamethyldisilazane, mounted on SEM stubs, sputter coated with gold-palladium, and examined with SEM.

TUNEL assays: Eye discs from wandering third instar larvae were dissected in PBS and fixed at room temperature in 2% formaldehyde in PBS for 10 min. Discs were rinsed with PBTE (PBS plus 1 mM EDTA and 0.2% Tween-20), digested with proteinase K (10 µg/ml) in PBS for 5 min at room temperature, rinsed with PBTE, refixed in 2% formaldehyde/PBS, and rinsed five times with PBS. TUNEL assays were performed with the Apo-BrdU TUNEL kit (Molecular Probes, Eugene, OR) according to manufacturer’s instructions. Labeled discs were mounted in PBS/50% glycerol plus Vectashield and analyzed by confocal microscopy as described above. As a control for apoptotic background using the EG/flu system, identically staged eye discs bearing FRT72B Nvmc clones were examined. No significant apoptosis above background was detected.

Actin filament-binding studies: Ovaries were dissected in PBS, rinsed once in modified G-PEM buffer plus 0.05% Tween-20 [80 mM PIPES, 1 mM MgCl2, 1 mM EGTA, 0.2 mM GTP, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 mM ptycetoxel, 1:100 dilution of phosphatase inhibitor and protease inhibitor cocktails (Sigma, St. Louis), and homogenized in 20 µl/ovary of the same buffer. Biotinylated phalloidin (Molecular Probes) was added to a final concentration of 0.15 units/ovary and incubated at room temperature with rotation for 30 min. Streptavidin-coated magnetic beads (Dynal, Great Neck, NY) were prepared by blocking for 30 min in PBS/5% BSA, rinsing, and resuspending in modified G-PEM buffer. Phalloidin-bound complexes were precipitated by adding blocked beads (0.08 µg/ovary) to the extracts and rotating at room temperature for 30 min. Beads were collected magnet-
bically, washed five times with PBS plus 0.05% Tween-20, and resuspended in 1/10 original volume of PBS. Complexes were then analyzed by immunoblotting according to standard protocols (Tolar and Pallanck 1998).

RESULTS

Identification and characterization of a Drosophila SCA2 homolog: To identify Drosophila homologs of the SCA2 gene, the human ataxin-2 protein sequence was used to query the BDGP database (Adams et al. 2000; Rubin et al. 2000). A single homolog of SCA2 (designated Datx2) was identified from this search. The Datx2 gene maps to polytene region 88F-89A1 of the third chromosome and encodes a polypeptide of 1084 amino acids exhibiting 23% amino acid identity and 36% amino acid similarity overall with human ataxin-2. Most of the sequence conservation between human ataxin-2 and Datx2 is confined to two domains (here designated ATX2-N and ATX2-C) that correspond to the most highly conserved sequences in the ataxin-2 family (Figure 1). Sequences outside these two domains are poorly conserved among ataxin-2 family members and share no homology to other known or predicted proteins. The ATX2-N domain of the ataxin-2 family contains Sm motifs, which are found in proteins involved in pre-mRNA splicing, snRNP biogenesis, and mRNA decapping (Mayes et al. 1999; He and Parker 2000; Tharun et al. 2000). The ATX2-N and ATX2-C sequences also align well to fully complements the ATX2-N and ATX2-C) that correspond to the most highly untranslated region (5′UTR) of SCA2 (Figure 1). Sequences outside these two domains are poorly conserved among ataxin-2 family members and share no homology to other known or predicted proteins. The ATX2-N domain of the ataxin-2 family contains Sm motifs, which are found in proteins involved in pre-mRNA splicing, snRNP biogenesis, and mRNA decapping (Mayes et al. 1999; He and Parker 2000; Tharun et al. 2000). The ATX2-N and ATX2-C sequences also align well to fully complements the ATX2-N and ATX2-C) that correspond to the most highly untranslated region (5′UTR) of SCA2 (Figure 1). Sequences outside these two domains are poorly conserved among ataxin-2 family members and share no homology to other known or predicted proteins. The ATX2-N domain of the ataxin-2 family contains Sm motifs, which are found in proteins involved in pre-mRNA splicing, snRNP biogenesis, and mRNA decapping (Mayes et al. 1999; He and Parker 2000; Tharun et al. 2000). The ATX2-N and ATX2-C sequences also align well to

The size discrepancy between the 140-kD band recognized by this antiserum and the 118 kD expected from theoretical translation of the Datx2 gene suggests that Datx2 migrates aberrantly on an SDS gel or that this protein is subject to post-translational modifications. Use of this antiserum to determine the Datx2 expression pattern in embryos revealed Datx2 protein in most tissues, with particularly high levels in the central nervous system (Figure 2C). Consistent with the subcellular localization of human ataxin-2, Datx2 localizes to the cytoplasm in all tissues examined, including neurons of the central nervous system and developing egg chambers (Figures 2, E and F, and 5A). Subcellular fractionation experiments using an adult head lysate confirmed the cytoplasmic localization observed in in situ analyses (data not shown).

Identification of Datx2 mutants:Datx2 genomic sequence was used to search a Drosophila database composed of sequences flanking known P-element transposon insertions (Spradling et al. 1995, 1999). This search identified three strains [designated l(3)06490, EP(3)3022, and EP(3)3145] bearing P-element insertions in the 5′-untranslated region (5′-UTR) of Datx2 (Figure 3). The l(3)06490 and EP(3)3022 insertions are inviable as homozygotes and fail to complement one another and the Df(3R)Po4 deletion that removes the Datx2 gene. Both insertions confer second instar larval lethality in trans to the Df(3R)Po4 chromosome. Precise excision of the l(3)06490 and EP(3)3022 P-elements reverted all phenotypes associated with these insertions. By contrast, the EP(3)3145 insertion is inviable as a homozygote, but fully complements the l(3)06490 and EP(3)3022 insertions and the Df(3R)Po4 deletion chromosome. Subsequent experiments revealed that the recessive lethality associated with the EP(3)3145 line results from a mutation unrelated to the EP(3)3145 insertion. This mutation was removed by recombination and the resulting homozygous viable line was designated EP(3)3145V. Unlike the l(3)06490 and EP(3)3022 insertions, the EP(3)3145 insertion contains a promoter designed to drive expression of flanking sequences and is oriented properly to drive expression of Datx2 (Rorth et al. 1998). Weak expression from the exogenous EP(3)3145 promoter may therefore drive sufficient levels of Datx2 expression to circumvent lethality.

To determine whether the recessive lethal phenotypes conferred by the l(3)06490 and EP(3)3022 insertions result from disruption of Datx2 function, transgenic lines consisting of a full-length Datx2 cDNA under GAL4 transcriptional regulation (Brand and Perrimon 1993) were generated and introduced into Datx2 mutant backgrounds. Surprisingly, the Datx2 transgenes were able to complement the l(3)06490 and EP(3)3022 recessive lethal phenotypes in the absence of GAL4 induction, presumably owing to leaky transgenic expression of Datx2 (Table 1; data not shown). These results demonstrate that the recessive lethal phenotypes of the l(3)06490 and EP(3)3022 insertions result from loss of Datx2 activity. Additional experiments using antisera to Datx2 revealed a dramatic reduction of Datx2 protein...
Figure 1.—Ataxin-2 homologs. Alignment of conserved amino acid sequences of human ataxin-2 (HATX2), human ataxin-2-related protein (HATX2RP), Drosophila melanogaster Datx2 (DATX2), C. elegans ATX-2 (CATX2), two ataxin-2 homologs from A. thaliana (AATX2a and AATX2b), and S. cerevisiae poly(A)-binding protein 1 (PBP1P). Amino acid similarities within the ATX2-N and ATX2-C domains shared by at least four ataxin-2 members are shaded. Amino acid identities within the ATX2-N and ATX2-C domains shared by at least six ataxin-2 family members are boxed. The Drosophila ataxin-2 polypeptide exhibits 43 and 62% amino acid identity to human ataxin-2 within the ATX2-N and ATX2-C domains, respectively. The Sm motifs within the ATX2-N domain are highlighted by large rounded boxes. The ATX2-C consensus sequence, representing positions of identity shared by four or more ataxin-2 family members, is shown in alignment with a conserved sequence present in peptides known to bind to the carboxyl terminus of PABP (designated the PAIP consensus). The polyglutamine tracts in the human and Drosophila ataxin-2 sequences are represented by black boxes. The peptide sequence used to generate the Datx2 antiserum is represented by the crosshatched box.
levels in $l(3)06490$ and $EP(3)3022$ homozygous tissues relative to wild-type controls (Figure 10A), further verifying that these $P$-element insertions specifically affect $Datx2$ function. For the remainder of this report the $l(3)06490$ and $EP(3)3022$ insertion strains are designated $Datx2^{l(3)06490}$ and $Datx2^{EP(3)3022}$, respectively, to denote that these insertions are alleles of the $Datx2$ gene.

To generate more severe alleles of $Datx2$, the viable $EP(3)3145V$ insertion was excised and imprecise excision alleles of the $P$ element were identified by failure
Datx2 and Datx2l(3)06490 alleles were characterized further. The Datx2X1 allele is a 1.4-kb deletion that removes the first 22 codons of the Datx2 coding sequence and extends into the first intron (Figure 3). The Datx2X2 allele is a 1.3-kb deletion, but this deletion does not extend into the Datx2 coding sequence. Both imprecise excision alleles confer second instar larval lethality in trans to a Datx2 deletion chromosome and this lethality can be rescued by transgenic expression of Datx2.

To compare the severity of the imprecise excision alleles to the Datx2 P element alleles, the Eyeless-GAL4 UASFLP/hid (EGUF/hid) system (Stowers and Schwarz 1999) was used to generate mosaic flies homozygous for the Datx2 mutations in the retina. Flies bearing retinal clones of the Datx2X1 or Datx2X2 alleles exhibit a mildly disorganized ommatidial pattern and a slight reduction in eye size (Figure 4B; data not shown). By contrast, flies bearing retinal clones of the Datx2X1 or Datx2X2 alleles exhibit a severe loss of eye tissue (Figure 4C; data not shown). These phenotypes could result from defective cell division, defective cell growth, cell death, or a combination of these factors. To begin to distinguish between these possibilities, eye discs from third instar larvae bearing Datx2 retinal clones were dissected and assayed for programmed cell death by TUNEL (Figure 4, D–F). Loss of Datx2 function increased the number of cells undergoing apoptosis, with more cells appearing positive for TUNEL in Datx2X1 retinal clones than in Datx2X2 retinal clones. In addition, eye discs from the Datx2X1 allele were smaller and manifested disorganized ommatidial structure. The increased apoptosis in Datx2 retinal clones indicates that the loss of eye tissue observed in adults results at least in part from apoptosis, although defects in cell division or growth might also play contributing roles. Given the severe molecular nature of the Datx2X1 allele and similar phenotypic characteristics of the Datx2X1 and Datx2X2 mutations, these mutations likely represent null alleles of the Datx2 gene. By contrast, the relatively milder phenotypes conferred by the Datx2X2/06490 and Datx2X2/3022 alleles indicate that these mutations are hypomorphic alleles of Datx2.

Datx2 is required for actin filament formation, oocyte specification, and oocyte positioning in the female germ line: The finding that Datx2 mutants bearing alleles of varying severities all exhibit second instar larval lethality, coupled with the large abundance of Datx2 transcripts at the earliest stages of embryonic development, suggested that early developmental requirements for Datx2 are supplied maternally. To investigate this possibility, Datx2 expression was analyzed in the female germ line. In Drosophila, oogenesis proceeds through a series of incomplete mitotic divisions of the germline lineage to yield an egg chamber consisting of 16 interconnected germ cells surrounded by a layer of somatically derived follicle cells. One of the 16 cells from the germline lineage proceeds through meiosis and becomes the future oocyte while the 15 remaining cells differentiate into supporting polyplid nurse cells (Spradling 1993). Results of immunocytochemical analysis indicate that Datx2 is present in nurse cells and the oocyte (Figure 5). Consistent with observations in other tissues (Figure 2E), immunoreactivity appeared to be exclusively cytoplasmic. Notably, increased Datx2 staining was observed in the oocyte and was enriched at the posterior cortex, confirming that a large maternal contribution of Datx2 is present during early embryogenesis.

To investigate the functional consequences of eliminating the maternal supply of Datx2, female flies bearing germline clones of the Datx2 mutations were examined. Germline clones of the Datx2X2/06490, Datx2X2, and Datx2X2 alleles were completely sterile and failed to lay eggs, whereas females bearing Datx2X2/3022 germline clones were also sterile, but occasionally laid small deformed eggs that failed to hatch. By contrast, female flies bearing germline clones of a precise excision derivative of

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<th>Rescue efficiency of UAS-Datx2 transgenes</th>
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<td>UAS-Datx2, 1B</td>
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<td>UAS-Datx2, 2.3</td>
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<td>UAS-Datx2, 2.4</td>
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*Rescue efficiency is defined as the percentage of homozygous Datx2X2/06490 mutants recovered from a cross of Datx2X2/06490 heterozygotes to UAS-Datx2, Datx2X2/06490 heterozygotes, relative to Mendelian expectations for full rescue. A minimum of 600 progeny were scored for each cross.
the Datx2^{l(3)06490} chromosome produced normal-looking eggs and were fertile. Furthermore, homozygous Datx2 mutants bearing a Datx2 rescuing transgene laid normal-looking eggs, at least some of which were capable of hatching, verifying that the egg-laying defect in Datx2 mutants derives from loss of Datx2 function.

To analyze the nature of the germline defect in Datx2 mutants, ovaries were dissected from females bearing Datx2 germline clones and stained with fluorescent phalloidin to highlight filamentous actin and 4′,6-diamidino-2-phenylindole (DAPI) to highlight cell nuclei. Most egg chambers from females with homozygous clones of the hypomorphic Datx2^{l(3)06490} and Datx2^{EP(3)3022} alleles are able to progress normally through the early and middle stages of oogenesis (Table 2; data not shown). However, most of these egg chambers arrest development at a stage in oogenesis when nurse cells rapidly transport their cytoplasmic contents into the oocyte through intercytoplasmic bridges termed ring canals that connect these cells to one another. The arrested egg chambers manifest enlarged nurse cells and a small poorly developed oocyte (Figure 6, C and I).

**Figure 4.**—Datx2 is required for normal eye development. (A) Compound eye from wild-type fly. (B) Compound eye from a fly homozygous for the Datx2^{l(3)06490} allele in the retina. Note the disorganized ommatidial structure and decreased size relative to the wild-type eye. Similar results were obtained with the Datx2^{2^{P(3)3022}} allele (data not shown). (C) Compound eye of a fly homozygous for the Datx2^{X1} allele in the retina. Note the dramatic decrease in size of the compound eye and lack of ommatidial structure. Identical results were obtained with the Datx2^{X2} allele (data not shown). (D–F) TUNEL assays for apoptosis in Datx2 mutant eye discs. Third instar eye discs from w^{118} control (D), Datx2^{l(3)06490} homozygous eye clones (E), and Datx2^{X1} homozygous eye clones (F) are shown. Apoptotic cells appear as bright spots indicated with arrows. The background was increased in D so that the eye disc could be seen in the absence of apoptotic cells. (G–I) Third instar larval eye discs stained with anti-Elav to compare eye disc size and ommatidial structure in the Datx2 mutants with respect to wild type. (G) w^{118} control. (H) Datx2^{l(3)06490} eye clone. (I) Datx2^{X1} eye clone.

**Figure 5.**—Datx2 is present in egg chambers and is enriched in the oocyte. (A) Wild-type egg chambers stained with Datx2 antiserum. Datx2 is distributed throughout the germline cytoplasm and is enriched in developing oocytes (arrows). (B) Wild-type egg chambers stained with preimmune serum. (C) Datx2 immunoreactivity is abolished in egg chambers derived from Datx2^{l(3)06490} germline clones (GLC). Note that staining in somatic follicle cells, which are heterozygous for the Datx2^{l(3)06490} mutation, is retained (arrows).
D). Normally, nurse cell nuclei are tethered by an actin filament cage that forms immediately prior to the cytoplasmic transport stage. Most of these anchoring filaments fail to form in the Datx2 mutants (Figure 6, E and F). In addition, the nurse cell nuclei are inappropriately positioned in close proximity to the ring canals and are sometimes observed stretching partially through the ring canals, indicating that the cytoplasmic transport failure in the Datx2 mutants results from occlusion of the ring canals by nurse cell nuclei (Figure 6, G and H). Mutations in genes regulating actin polymerization and bundling also disrupt the formation of this cage and result in cytoplasmic transport defects identical to those seen in Datx2 mutant egg chambers (Peifer et al. 1995; reviewed by Robinson and Cooley 1997). These results suggest that actin polymerization or bundling defects are responsible for the cytoplasmic transport failure in the Datx2°(3)06490 and Datx2°(3)0122 mutants.

Although most Datx2° egg germline stem cells appear to undergo normal cell division to yield the 16-cell cystoblast (Table 2), all of these egg chambers arrested development prior to the cytoplasmic transport stage. Normally the oocyte resides at the posterior end of the egg chamber, adjacent to the posterior somatic follicle cells. However, in egg chambers homozygous for the Datx2° allele, the oocyte was often found in the middle of the egg chamber or the oocyte failed to be specified at all (Table 2; Figure 6, I–L). Oocyte contact with the posterior follicle cells is required to prevent these cells from pursuing their default anterior state (Godt and Tepass 1998). As a consequence, oocyte mispositioning in the Datx2° egg chambers often results in egg chambers with two anterior ends (Figure 6L). Despite these defects, however, several aspects of egg chamber development appear to proceed normally. For example, the oocyte induces surrounding somatic follicle cells to become columnar in shape, and this process appears to be intact in Datx2° egg chambers. (Figure 6, K and L). Furthermore, the border cells, which normally delaminate from the follicular epithelium and migrate toward the oocyte under direction of a signal emanating from the oocyte, migrate toward the mispositioned oocyte in Datx2° egg chambers, demonstrating that this process is also intact. Because proper cell adhesion between the oocyte and the posterior somatic follicle cells is required for the oocyte to maintain its posterior position in the egg chamber (Godt and Tepass 1998), the oocyte mispositioning defect suggests a possible requirement for Datx2 in mediating cell adhesion. Females bearing germline clones of hypomorphic Datx2 alleles produced egg chambers with some of these same defects, but much less frequently (Table 2).

**Reduced Datx2 activity results in bristle defects:** In the absence of GAL4-induced expression, several UAS-Datx2 transgenes are capable of rescuing the Datx2 larval lethality (Table 1). Rescued adult flies display several defects, however. Datx2 mutants rescued by the UAS-Datx2.1B transgene are uncoordinated, display a rough eye phenotype like that of flies bearing retinal clones of the Datx2°(3)06490 allele, and exhibit bent and forked thoracic sensory bristles. These phenotypes are fully penetrant in flies rescued by this transgene, although typically only a small number of the bristles exhibited gross structural alterations, with the scutellar, humeral, and sternopleural bristles being primarily affected. Datx2 mutants rescued by the UAS-Datx2.2.3 and UAS-Datx2.4 transgenes exhibit normal motor behavior and eye structure, but continue to manifest bent and forked sensory bristles (Figure 7, A and B), although the penetrance of the sensory bristle phenotypes was reduced in these flies. These phenotypes appear to arise as a consequence of reduced Datx2 function rather than overexpression or misexpression of Datx2 because these same transgenes do not produce detectable phenotypes.

### Table 2

**Summary of Datx2 female germline clone phenotypes**

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<tr>
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<th>Normal precytoplasmic dumping egg chambers</th>
<th>Normal postcytoplasmic dumping egg chambers</th>
<th>Mispositioned oocyte</th>
<th>Oocyte specification failure</th>
<th>Degenerating egg chambers</th>
<th>Extra germline cells</th>
<th>Fewer germline cells</th>
<th>Cytoplasmic dumping failure</th>
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<tr>
<td>Datx2°(3)06490° (control)</td>
<td>72</td>
<td>27</td>
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<td>0</td>
<td>1</td>
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<tr>
<td>Datx2°(3)06490°</td>
<td>56</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
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<td>42</td>
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<td>10</td>
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<td>N/A</td>
</tr>
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Tallies are the distribution percentage of all egg chambers produced by these females. NA, not applicable.

1 Includes all egg chambers prior to the cytoplasmic dumping stage.

2 Includes degenerating egg chambers from all stages.

3 Datx2°(3)06490° represents a precise excision derivative of the l(3)06490 Datx2 insertion. This precise excision allele was used to generate germline clones using the same procedure used for the Datx2°(3)06490 and Datx2° alleles.

4 Percentages do not total 100% due to rounding.

5 No egg chambers from Datx2° clones progress to the cytoplasmic dumping stage.
in a wild-type background. Moreover, the phenotypes in partially rescued Datx2 mutants do not result from other recessive mutations on the chromosomes bearing the Datx2 mutations, as these phenotypes are observed in flies bearing independently generated Datx2 mutations.

Sensory bristle development depends initially on the assembly of parallel bundles of actin filaments arranged in repeated units (Tilney et al. 1996). These bundles serve as a temporary support scaffold for the subsequent deposition of cuticle. Following cuticle deposition, the actin filament scaffold is disassembled. The parallel ridge pattern of the adult bristle is thus a reflection of the parallel actin bundle organization in the developing bristle. Many of the bristles in partially rescued Datx2 mutants exhibit highly disorganized cuticular ridges, indicating that the actin filament scaffold in the developing bristle was improperly assembled (Figure 7, C and D).

To examine the structure of the actin scaffold in the developing bristle, thoraces from 48-hr-old pupae were dissected, and actin filaments in developing bristles were examined by staining with fluorescent phalloidin conjugates. In wild-type bristles, the individual units of bundled fibers and the stereotypical arrangement of transverse breaks in these bundles were apparent (Figure 7E). However, in partially rescued Datx2 mutants and flies bearing clones of the Datx2X1 allele, the actin filament bundle repeat unit is significantly shorter and heterogeneous in length, leading to discontinuities in the transverse breaks of actin bundles (Figure 7, F–H). Although the lengths of these bundles are shorter than those from wild-type flies, most of the bundles are assembled of nurse cell nuclei (arrows) compared to the wild type. (C) Wild-type egg chamber after cytoplasmic transport has occurred. Note the increase in oocyte volume (denoted by dashed line) and concomitant decrease in nurse cell volume. (D) Datx2<l>3(06490</l> mutant egg chamber after the cytoplasmic transport stage. Note large volume of nurse cells and small volume of oocyte (denoted by dashed line) relative to wild type. (E) Confocal image of phalloidin-stained wild-type egg chamber immediately prior to cytoplasmic dumping. Note prominent array of cytoplasmic actin filaments. (F) Confocal image of phalloidin-stained Datx2<l>3(06490</l> mutant egg chamber just prior to cytoplasmic transport. Note the decreased density of actin filaments compared to wild type. (G and H) Magnified views of two different Datx2<l>3(06490</l> mutant egg chambers showing nurse cell nuclei stretching toward and through cytoplasmic bridges (ring canals) connecting nurse cells to the oocyte (arrows). (I and J) Confocal images of early stage egg chambers stained with DAPI to highlight nuclei. (I) Wild-type egg chamber with 15 nurse cells (numbered) and an oocyte (oc). (J) Datx2<l>3(06490</l> mutant egg chamber with 16 nurse cells (numbered). (K and L) Confocal images of early stage egg chambers stained with phalloidin. (K) In wild-type egg chambers, columnar follicle cells surround the oocyte at the posterior end (denoted by dashed line) and border cells (bc) can be seen delaminating from the follicular epithelium and migrating toward the oocyte. (L) In the Datx2<l>3(06490</l> mutant egg chamber the oocyte (oc) is located near the middle of the egg chamber. Note the presence of columnar follicle cells (dashed lines) in the vicinity of the misplaced oocyte and migration of border cells (bc) toward the misplaced oocyte.

Figure 6.—Datx2 is required for actin filament formation, oocyte specification, and oocyte positioning in the female germline. (A–D) Egg chambers stained with DAPI (blue) and fluorescently labeled phalloidin (red) to highlight cell nuclei and filamentous actin, respectively. (A) Wild-type egg chamber immediately prior to cytoplasmic transport. (B) Arrested Datx2<l>l(3)06490</l> mutant egg chamber. Note irregular arrangement of nurse cell nuclei (arrows) compared to the wild type. (C) Wild-type egg chamber after cytoplasmic transport has occurred. Note the increase in oocyte volume (denoted by dashed line) and concomitant decrease in nurse cell volume. (D) Datx2<l>3(06490</l> mutant egg chamber after the cytoplasmic transport stage. Note large volume of nurse cells and small volume of oocyte (denoted by dashed line) relative to wild type. (E) Confocal image of phalloidin-stained wild-type egg chamber immediately prior to cytoplasmic dumping. Note prominent array of cytoplasmic actin filaments. (F) Confocal image of phalloidin-stained Datx2<l>3(06490</l> mutant egg chamber just prior to cytoplasmic transport. Note the decreased density of actin filaments compared to wild type. (G and H) Magnified views of two different Datx2<l>3(06490</l> mutant egg chambers showing nurse cell nuclei stretching toward and through cytoplasmic bridges (ring canals) connecting nurse cells to the oocyte (arrows). (I and J) Confocal images of early stage egg chambers stained with DAPI to highlight nuclei. (I) Wild-type egg chamber with 15 nurse cells (numbered) and an oocyte (oc). (J) Datx2<l>3(06490</l> mutant egg chamber with 16 nurse cells (numbered). (K and L) Confocal images of early stage egg chambers stained with phalloidin. (K) In wild-type egg chambers, columnar follicle cells surround the oocyte at the posterior end (denoted by dashed line) and border cells (bc) can be seen delaminating from the follicular epithelium and migrating toward the oocyte. (L) In the Datx2<l>3(06490</l> mutant egg chamber the oocyte (oc) is located near the middle of the egg chamber. Note the presence of columnar follicle cells (dashed lines) in the vicinity of the misplaced oocyte and migration of border cells (bc) toward the misplaced oocyte.
Figure 7.—Datx2 is required for normal bristle morphology. Scanning electron micrographs of thoracic bristles from wild-type flies (A and C) and from Datx2^{21} mutants rescued with the UAS-Datx2.4 transgene (B and D) are shown. Wild-type bristles exhibit a regular alignment of cuticular ridges (C) and taper to a tip (A). Thoracic bristles from partially rescued Datx2 mutants are often split (B) and exhibit twisted and irregular cuticular ridges (D). (E–H) Confocal micrographs of phalloidin-stained bristles from 48-hr-old pupae. Actin bundles in wild-type flies are homogeneous in size and exhibit regular transverse breaks (E) whereas actin bundles from Datx2^{21} mutants rescued with the UAS-Datx2.4 transgene (F) or from Datx2^{21} clones (G and H) were shorter and thinner than those from wild type. Note the irregular spacing of transverse gaps in bundled filaments relative to wild type. (I and J) Scanning electron micrographs of bristles from wild-type fly (I) and from a fly ubiquitously overexpressing Datx2 (J). Note the increased bending and ribbon-like appearance in the fly overexpressing Datx2 (J). Bars: A–H, 5 μm; I and J, 50 μm.

parallel to the long axis of the growing bristle. Thin bundles of actin filaments were also observed. These results indicate that the defective bristle morphology observed in partially rescued Datx2 mutant adults arises from defective actin filament structure during bristle development.

Datx2 function is dosage sensitive: To determine tissue-specific requirements for Datx2, we attempted to rescue the Datx2 bristle, eye, and behavioral phenotypes resulting from reduced Datx2 activity by using the GAL4/UAS system (Brand and Perrimon 1993) to induce elevated Datx2 expression in specific tissues. In each of these experiments, however, the increased Datx2 expression conferred by GAL4 resulted in overexpression phe-
notypes, precluding a detailed analysis of Datx2 tissue-specific requirements (Table 3). For example, ectopic expression of Datx2 in the retina using GMR-GAL4 (Ellis et al. 1993) produces a range of eye phenotypes, with the UAS-Datx2.1B transgene producing progressive loss of pigmentation, and the UAS-Datx2.3 and UAS-Datx2.4 transgenes producing more severe disruptions of retinal structure (Figure 8). Ectopic expression of Datx2 in the nervous system using the elav-GAL4 (Robinow and White 1988) or eyeless-GAL4 drivers (Callaerts et al. 2001) or in mesoderm using the 24B-GAL4 driver (Brand and Perrimon 1993) results in significant developmental delays and lethality (Table 3). Weak ubiquitous expression of Datx2 using a heat-shock promoter-driven GAL4 line in the absence of heat shock results primarily in lethality with rare survivors exhibiting thin and somewhat flattened bristles (Figure 7, I and J). The severity of the phenotypes resulting from overexpression of Datx2 correlates with the levels of Datx2 expression conferred by the UAS-Datx2 transgenes (data not shown). These results indicate that Datx2 function is dosage sensitive.

Altered Datx2 dosage in the retina results in aberrant actin structures: Since altered Datx2 dosage is associated with actin filament formation defects in ovaries and bristles, we investigated whether altered Datx2 function also elicits cytoskeletal defects in the nervous system. This analysis was performed by comparing eye imaginal discs from wild-type flies, Datx2 mutants, and flies overexpressing Datx2. Third instar larval eye discs from wild-type Drosophila exhibit ommatidial cell clusters posterior to the morphogenetic furrow (rosettes) consisting, in part, of photoreceptor precursor cells with filamentous actin distributed near the subcortical regions of the individual cells within the clusters (Figure 9A). By comparison, the arrangement of filamentous actin in rosettes appears disorganized in similarly staged eye discs from mosaic larvae lacking Datx2 in the retina, and punctate accumulations of actin are seen throughout these clusters (Figure 9B). Overexpression of Datx2 in the retina using a UAS-Datx2 transgene in conjunction with GMR-GAL4 results in similar punctate actin aggregates and disorganized actin clusters (Figure 9C). Together, these results demonstrate that proper Datx2 expression levels are critical for regulating the morphology of actin structures in prephotoreceptor cells.

Datx2 does not regulate actin abundance or physically associate with filamentous actin: To determine whether the actin filament formation defects associated with altered Datx2 dosage result from changes in actin abundance, protein extracts were prepared from germline clones of Datx2 mutant ovaries and steady-state actins were analyzed by Western blot analysis. Although Datx2 abundance was markedly reduced in extracts from mutant ovaries, actin abundance remained essentially unchanged (Figure 10A). Actin abundance in flies overexpressing Datx2 was similarly unaffected (data not shown). These results indicate that the Datx2 phenotypes do not result from defective actin synthesis and suggest instead that Datx2 function is required for mobilization of actin monomers into filaments or bundles.

To investigate the possibility that Datx2 coordinates actin filament formation by assembling with actin filaments, a biochemical approach was used to test for
Figure 8.—Overexpression of Datx2 during eye development is toxic. Wild-type flies expressing Datx2 in the retina under control of the GMR-GAL4 driver (C–F) exhibit rough eyes, disorganized ommatidia, and loss of pigment cells relative to control flies bearing only the GMR-GAL4 driver (A and B) or flies bearing only a UAS-Datx2 transgene (data not shown). The degree of retinal disruption depends on the Datx2 transgenic line used and the age of the fly. Flies expressing Datx2 in the retina from a weakly expressing Datx2 transgene (UAS-Datx2.1B) display a mild rough eye phenotype at 1 day (C), progressing to loss of eye pigmentation by 4 weeks of age (D). Control flies bearing only the GMR-GAL4 driver at 1 day and 4 weeks (A and B, respectively) or the UAS-Datx2.1B transgene alone (data not shown) at the same time points exhibit normal eyes. Stronger Datx2 transgenic lines (UAS-Datx2.4 and UAS-Datx2.3) result in severe disruption of eye structure (E and F, respectively).

Figure 9.—Altered Datx2 gene dosage triggers the formation of aberrant filamentous actin structures in eye discs. The field shown is posterior to the morphogenetic furrow and is at a similar developmental stage. (A) Confocal projection of wild-type third instar eye disc stained with fluorescently labeled phalloidin to highlight filamentous actin. Rosettes are indicated with arrows. (B) Eye disc fully homozygous for the Datx2<sup>2P106490</sup> mutation stained with phalloidin. Note the bright, punctate actin-staining structures in the cells of the rosette (arrows). (C) Eye disc from a fly ectopically expressing Datx2 from the UAS-Datx2.3 transgene under the direction of GMR-GAL4. Like the Datx2 loss-of-function discs, actin staining was punctate and disorganized in these discs as a result of Datx2 overexpression (arrows). Bars, 5 μm.

physical interactions between Datx2 and actin filaments. Filamentous actin was precipitated from ovarian extracts using biotinylated phalloidin. Although much of the actin in these extracts was precipitated by phalloidin, all of the Datx2 was found in the soluble fraction (Figure 10B). Further subcellular fractionation experiments and in vitro actin filament-binding studies confirmed these results (data not shown). These experiments demonstrate that Datx2 is not a structural component of actin filaments and suggest that Datx2 regulates actin filament formation pathways through an indirect mechanism.

DISCUSSION

Spinocerebellar ataxia type 2 is a dominantly inherited neurodegenerative disorder caused by CAG expan-
in the coding sequence of the SCA2 gene (Imbert et al. 1996; Pulst et al. 1996; Sanpei et al. 1996). As with many of the polyglutamine repeat diseases, the mechanism of SCA2 pathogenesis and the normal cellular function of the corresponding gene are unknown. In mediate actin filament formation or bundling. The most conclusive evidence for a role of Datx2 in actin filament metabolism derives from studies of germline clones of Datx2 hypomorphic alleles and from analyses of bristle structure in Datx2 mutants. Females with germlines homozygous for hypomorphic Datx2 alleles are sterile owing to a reduction in nurse cell cytoplasmic actin filaments and an ensuing nurse cell cytoplasmic transport defect. This phenotype is nearly identical to the germline phenotypes in the Drosophila mutants chickadee, quail, and singed (reviewed by Robinson and Cooley 1997). chickadee encodes a Drosophila homolog of profilin, which recruits actin monomers into polymerized microfilaments (Verheyen and Cooley 1994), and quail and singed encode the microfilament-bundling proteins villin and fascin, respectively (Cant et al. 1994; Mahajan-Miklos and Cooley 1994). The similar phenotypes produced by these mutants suggest that Datx2 mutants may regulate or function in parallel with these gene products in the ovary. Notably, the chickadee and singed mutations also result in bristle phenotypes similar to those of the partially rescued Datx2 mutants (Cant et al. 1994; Mahajan-Miklos and Cooley 1994). These bristle phenotypes also derive from defective actin filament formation and bundling in the developing bristle. Furthermore, mutations in a number of other genes involved in actin filament formation and bundling pathways produce similar sensory bristle anomalies (Hoppmann et al. 1996; Chen et al. 2001; Grieshaber et al. 2001). Like these mutants, the aberrant actin bundles in Datx2 mutants likely compromise the strength or stiffness of the scaffold, resulting in insufficient support during cuticle deposition. Subsequent buckling of the underlying actin scaffold may thus be responsible for the disorganized ridges and bent and forked bristles observed in Datx2 mutants. The actin filament formation defects observed in Datx2 mutants do not appear to result from reduced actin synthesis since actin levels are not significantly affected by altered Datx2 dosage. Rather, phenotypic similarities between Datx2, chickadee, quail, and singed suggest that, like these factors, Datx2 mediates actin filament formation or bundling.

The oocyte mispositioning defect observed in the Datx20 allele egg chambers might also result from actin filament formation defects. For example, germline clone analysis of particular chickadee alleles produces oocyte mispositioning phenotypes similar to those seen in Datx20 germline clones (Peifer et al. 1993). Furthermore, the Drosophila β-catenin homolog (armadillo) has been shown to function by anchoring the cell adhesion molecule DE-cadherin to the subcortical actin cytoskeleton (Bart et al. 1997). Like the Datx20 allele, null alleles of armadillo
also result in egg chambers with mispositioned oocytes (Peifer et al. 1993). Defects in the subcortical actin cytoskeleton of Datx2 egg chambers might therefore phenocopy armadillo mutants by interfering with Dé-cadherin attachment to the subcortical cytoskeleton. While the oocyte mispositioning defect can be related to actin filament defects, a precise interpretation of the oocyte specification defect observed in Datx2°/° egg chambers is complicated by the somewhat limited understanding of oocyte specification mechanisms, and further experimentation will be required to elucidate the role of Datx2 in early egg chamber development.

Consistent with our observations in bristles and the female germline, altered Datx2 dosage in the nervous system also affected actin filament structure. Prephotoreceptor neurons homozygous for the Datx2 mutations or overexpressing Datx2 from a transgene exhibited punctate, disorganized aggregates of actin. An increase in the frequency of apoptosis accompanied these actin filament formation defects. Both phenotypes were observed early in development of the photoreceptor neurons and occur before any other visible signs of gross cellular dysfunction, indicating that the eye phenotypes resulting from altered Datx2 dosage derive from perturbations in actin filament formation and/or apoptosis. The coincident occurrence of actin filament formation defects and apoptosis in prephotoreceptor neurons raises the possibility that the anomalies in filamentous actin-containing structures are secondary consequences of previous apoptotic events. However, we favor the idea that apoptosis is triggered in response to defective actin filament formation or bundling, since most retinal cells with altered Datx2 dosage exhibited filamentous actin anomalies while relatively few were positive for apoptosis. Furthermore, apoptosis was not observed in egg chambers prior to the defects in actin morphology, nor was it observed in epithelial cells making bristles (data not shown). The increased levels of apoptosis in Datx2°/° eye disc clones relative to eye clones from the hypomorphic Datx2 alleles suggest that apoptosis is at least partly responsible for the severe eye phenotype in these mutants. These data suggest that Datx2-mediated perturbations in actin filament formation trigger apoptosis of retinal cells.

The finding that Datx2 overexpression was toxic in all tissues tested suggests that the pathways regulated by Datx2 are dosage sensitive. Alternatively, excessive production of Datx2 protein could be toxic for reasons unrelated to the normal cellular function of this protein. Two observations suggest that the overexpression phenotypes stem from perturbation of pathways normally regulated by Datx2. First, the disorganized actin structures observed in the developing retinas of flies overexpressing Datx2 closely resemble those seen in retinal clones of the Datx2 loss-of-function alleles, suggesting that the Datx2 overexpression phenotypes also derive from perturbation of the actin cytoskeleton. Second, as is seen in partially rescued Datx2 mutants, ubiquitous overexpression of Datx2 results in defective bristle morphology, again suggesting that overexpression of Datx2 perturbs actin filament polymerization or bundling. Together, these results argue that the Datx2 overexpression phenotypes result from perturbation of the actin cytoskeleton and, thus, that the normal cellular role played by Datx2 is dosage sensitive.

Conserved sequence motifs in the ataxin-2 family and experiments with several ataxin-2 homologs suggest that these proteins function in RNA metabolism (Mangus et al. 1998; Shibata et al. 2000; Kozlov et al. 2001). In particular, the ATX2-C domain is highly conserved with sequences known to mediate interactions with PABP. Studies conducted with the yeast ataxin-2 protein, Pbp1p, have shown that this protein interacts physically and functionally with PABP (Mangus et al. 1998; Kozlov et al. 2001). Yeast two-hybrid experiments carried out with human ataxin-2 have identified an ataxin-2-binding protein (A2BP) containing RNA recognition motifs (Shibata et al. 2000). The existence of a highly conserved Drosophila homolog of the A2BP protein (Adams et al. 2000; data not shown), suggests that this interaction is conserved in flies. Given these findings and our experiments showing that Datx2 fails to colocalize with actin filaments, we suggest that Datx2 functions by regulating the expression of a subset of transcripts encoding mediators of actin polymerization or bundling. Alternatively, Datx2 may function in a more general capacity to regulate expression of a broad class of transcripts, so that the phenotypes resulting from altered Datx2 dosage represent those of the most dosage-sensitive genes. Although definitive evidence distinguishing these two models will require further work, two lines of evidence lead us to favor the former possibility. First, Drosophila mutants with compromised translational capacity exhibit short thin bristles with reduced ridging (Sørbøe Larsen et al. 1998; Korey et al. 2001), whereas the bristles in Datx2 mutants are bent and forked with disorganized ridging and thus more closely resemble those produced by mutations specifically affecting actin filament formation pathways (Cant et al. 1994; Mahajan-Miklos and Cooley 1994; Hopmann et al. 1996; Chen et al. 2001; Grieshaber et al. 2001). Second, altered Datx2 dosage does not appear to significantly affect the overall abundance of actin, demonstrating that altered levels of this potentially limiting component do not account for the Datx2 phenotypes. Thus, we favor a model whereby Datx2 regulates a subset of RNAs, at least some of which encode mediators of actin filament formation. This regulation could be at the level of transcript stability, transcript localization, or translational regulation, and the molecular basis of this regulation is the focus of current experimentation.

Previous work on the polyglutamine disorders has led to a model whereby nuclear localization of long polyglutamine repeats induces neurodegeneration
through a transcriptional interference mechanism (La Spada et al. 2001; Shimohata et al. 2001; Steffan et al. 2001). While solid evidence supports this model for several of the polyglutamine disorders, recent work has shown that nuclear localization is not a prerequisite for SCA2 pathology, indicating a distinct mechanism of pathology for this disorder (Huynh et al. 2000). Our finding that altered dosage of Dats2 is associated with cytoskeletal alterations that result in loss of degeneration of tissues suggests the possibility that cytoskeletal defects resulting from polyglutamine-mediated alteration of ataxin-2 activity are responsible for SCA2 pathology. This hypothesis is consistent with the cytoplasmic localization of ataxin-2 protein and is supported by the observation that loss of dendritic structure, which is at least partly dependent upon actin microfilament metabolism, represents an early event in SCA2 pathogenesis (Huynh et al. 2000). Further support for such a mechanism of SCA2 pathology is provided by studies demonstrating the importance of the actin cytoskeleton for neuronal development and maintenance (Li et al. 2000; Minamide et al. 2000; Narayama et al. 2000) and by the growing number of examples of neurodegenerative disorders caused by mutations in genes encoding cytoskeletal components or regulators of cytoskeletal maintenance (Julien and Beaulieu 2000; McMurray 2000; Brenner et al. 2001; García and Cleveland 2001).

In summary, our results suggest the hypothesis that polyglutamine expansion of human ataxin-2 alters the normal cellular function of this polypeptide, resulting in the pathological consequences observed in SCA2 individuals. While this hypothesis represents a departure from the predominant model to explain pathology in the polyglutamine disorders, recent work suggests that polyglutamine-mediated alteration of the normal cellular functions of the genes underlying SCA6 and HD may be responsible for pathogenesis in these disorders (Dragatsis et al. 2000; Restituito et al. 2000). It is also clear from studies of DRPLA, SCA1, SCA6, and AR, the only polyglutamine disease genes encoding proteins with defined functions, that polyglutamine expansion directly affects the normal activities of the encoded gene products (Tut et al. 1997; Restituito et al. 2000; Yue et al. 2001; Zhang et al. 2002), raising the possibility that alteration of the cellular functions of these proteins contributes to pathogenesis in these disorders. A more detailed understanding of Dats2 function, particularly its role in the nervous system, will provide the foundation for a direct test of our hypothesis. Availability of Dats2 loss-of-function and overexpression phenotypes, coupled with the tools available for genetic analysis in Drosophila, will allow rapid progress toward this goal.

We are indebted to the BDGP and Bloomington Drosophila stock center for Dats2 sequence information and Drosophila stocks used in this work. We thank C. Berg for advice on ovary dissection and staining and Saverio Brogna for antisem for ADH. We thank Jessica Greene, Rashmi Dayalu, and Sophie Waliany for technical support.

We are grateful to B. Wakimoto and all members of the Pallanck lab for critical analysis of this manuscript. Finally, we thank the Electron Microscopy Shared Resource laboratory of the Fred Hutchinson Cancer Research Center for conducting scanning electron microscopic analysis.

This work was supported by National Institutes of Health grants 5K01 DK02706 (to S.M.J.) and GM07735-23 (to T.F.S.).

LITERATURE CITED


