p53 prevents neurodegeneration by regulating synaptic genes

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DNA damage has been implicated in neurodegenerative disorders, including Alzheimer's disease and other tauopathies, but the consequences of genotoxic stress to postmitotic neurons are poorly understood. Here we demonstrate that p53, a key mediator of the DNA damage response, plays a neuroprotective role in a *Drosophila* model of tauopathy. Further, through a whole-genome ChIP-chip analysis, we identify genes controlled by p53 in postmitotic neurons. We genetically validate a specific pathway, synaptic function, in p53-mediated neuroprotection. We then demonstrate that the control of synaptic genes by p53 is conserved in mammals. Collectively, our results implicate synaptic function as a central target in p53-dependent protection from neurodegeneration.

p53 transcriptional function | ChIP-chip | neurodegeneration | tauopathy | synaptic genes

Neurodegenerative diseases are a group of inexorably progressive disorders characterized by ongoing dysfunction and death of postmitotic neurons and are among the principal causes of morbidity and mortality associated with aging (1–3). Tauopathies, including Alzheimer's disease, are defined neuropathologically by aggregation and deposition of the microtubule-associated protein tau accompanying neuronal loss (1–3). DNA damage has been documented in neurodegenerative disorders, including tauopathies, but the effects of DNA damage on the function and viability of nondividing neurons are poorly understood (4–6).

In this study, we used a *Drosophila* model of tauopathy based on expression of human tau carrying the R406W mutation, which is found in patients with the familial tauopathy frontotemporal dementia with Parkinsonism linked to chromosome 17 (7). Our model recapitulates several key features of human tauopathies, including accumulation of abnormally phosphorylated and conformationally altered tau, age-dependent neurodegeneration, and early death (7). We have previously shown that expression of human tau in *Drosophila* neurons induces the DNA damage response, including p53 up-regulation, and that reducing the function of the DNA damage checkpoint significantly increases neurodegeneration (5). These data suggest an unexpected neuroprotective role for p53 in postmitotic neurons. We were therefore motivated to examine the transcriptional function of p53 in the context of progressive neurodegeneration.

p53, together with p63 and p73, comprises a family of transcription factors, which regulate fundamental processes including proliferation, differentiation, senescence, and cell death (8, 9). The p53 family has been extensively studied in tumorigenesis, but the role of family members in postmitotic neurons is poorly defined, with an emerging body of literature suggesting involvement of the p73 subfamily in aspects of neuronal development and neurodegeneration (10–12). Using *Drosophila* to study p53-dependent transcription in neurodegeneration has two significant advantages. First, transcription can be studied in aging neurons in vivo. Second, analysis of overall p53 family function in a simplified genome, which contains a single *p53* gene, may reveal "ancestral" activities of the family (9, 13).

Here we show that p53 is neuroprotective in an in vivo model of tauopathy. Through chromatin immunoprecipitation (ChIP)-chip analyses we determine that p53 controls the transcription of a group of genes involved in synaptic function. Genetic manipulation of these genes modifies tau neurotoxicity. We find that the transcriptional control by p53 of these synaptic genes is conserved in murine neurons and human brain. Our results thus implicate synaptic function as a critical p53 target in neuroprotection.

Results

p53 Protects from Neurodegeneration During Aging in Vivo. To investigate the role of p53 in tauopathy pathogenesis, we first expressed tau in flies lacking p53 (Fig. 1*A*). Although *p53*-null flies showed no evidence of neurodegeneration in the absence of transgenic human tau (Fig. 1 *B–D*), cell death, as measured by the number of TUNEL-positive neurons, significantly increased in the brains of tau-expressing, *p53* ($p53^{11\text{-}1B\text{-}1}$)-null animals compared with tau-transgenic animals with normal levels of endogenous p53 (Fig. 1*B* and Fig. S1*A*, *Left*). Neurodegeneration in our model, as in mouse tauopathy models and in Alzheimer's disease, is associated with abnormal reactivation of the cell cycle in postmitotic neurons (Fig. S1*B*) (5, 6, 14, 15). Removing p53 from tau-transgenic flies significantly increased cell-cycle reactivation, as measured by proliferating cell nuclear antigen (PCNA) reexpression in neurons (Fig. 1*C* and Fig. S1*A*, *Right*). Enhancement of human tau-driven neuronal death and cell-cycle reactivation was also observed when a second *p53* null allele ($p53^{5A\text{-}1\text{-}4}$; Fig. S1 *C* and *D*) was used to remove p53 function. To

Significance

The transcription factor p53 plays a critical role in the cellular response to DNA damage and has thus been studied intensively in oncogenesis research. However, the role that p53 plays in the response of postmitotic neurons to cellular stress has received less attention. Here we describe an unexpected neuroprotective role for p53 in an in vivo model of tau-mediated neurodegeneration relevant to Alzheimer's disease and related disorders. Further, we identify synaptic function as a novel target pathway for p53 in aging neurons, consistent with the growing evidence for synaptic pathology as an early event in neurodegenerative disease. Our study defines mechanistically a new, conserved role for p53 in protecting postmitotic neurons from degeneration during aging and disease.

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The authors declare no conflict of interest.

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Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE40418).

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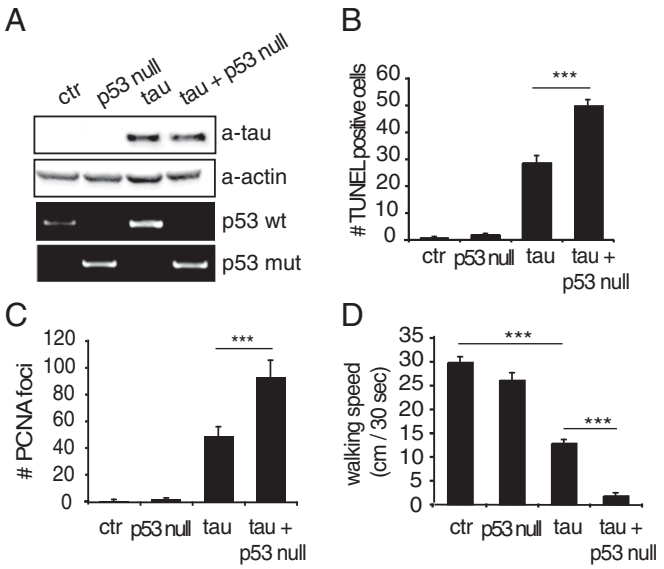

Fig. 1. p53 is neuroprotective in *Drosophila* tauopathy. (*A*, *Upper*) Tau protein levels do not change in *p53*-null flies. (*Lower*) Genomic PCR analysis confirms genotypes at the *p53* locus for the animals used. (*B*) Apoptosis, indicated by TUNEL-positive neurons, increases in the brains of tau-transgenic, *p53*-null animals. (*C*) Cell-cycle reactivation, indicated by PCNA-positive foci, increases in the brains of tau-transgenic, *p53*-null animals. In *B* and *C*, flies are 10-d-old ($n \geq 6$ per genotype). (*D*) Removing p53 in tau-transgenic animals worsens locomotion, measured as walking speed. Flies are 2-d-old ($n = 18$ per genotype). In the graph, values represent mean ± SEM. Asterisks indicate significant differences compared with control by ANOVA with Student–Newman–Keuls post hoc test for multiple comparisons. ***$P <$ 0.001. ctr, control: *elav-GAL4*/+.

evaluate the effect of p53 deficiency on an additional measure of nervous system integrity, we assessed locomotor function. We found that the locomotor defect present in tau-transgenic flies, as assayed by measuring walking speed, was significantly worsened by removing p53 (Fig. 1*D*) (16). Importantly, removing p53 did not influence tau expression (Fig. 1*A*). Together, these results support a neuroprotective role for p53 in our tauopathy model.

ChIP-chip Analysis of p53 Targets in Tauopathy. To delineate, on a global scale, the transcriptional targets in p53-mediated neuroprotection, we performed a genome-wide ChIP-chip analysis. To provide a positive control and test the specificity of the *Drosophila* p53 antibody, we first determined by traditional ChIP whether p53 protein binds its own gene promoter in *Drosophila,* as it does in mammals (17). The *Drosophila p53* gene has two putative p53 regulatory elements upstream of the P2 promoter, and we designed primers that would amplify this region specifically (Fig. 2*A*, *Lower*). As a control for p53 immunoprecipitation, we used *p53*-null flies. We found that p53 protein bound its own promoter with an enrichment 12 times greater than a region in the 3′ UTR with no p53 regulatory elements (Fig. 2*A*, *Upper*). In addition, we demonstrated the specificity of the p53 antibody for the p53 protein by performing a ChIP assay in fly embryos. In this assay, we confirmed that p53 binds the promoter of the *p53* and *rpr* genes and that p53 binding increases substantially after irradiation (Fig. S2), consistent with prior reports (18).

Having thus demonstrated the efficiency and specificity of our *Drosophila* p53 ChIP assay, we performed ChIP-chip experiments. We used chromatin precipitated by the anti-p53 antibody in aged control and tau-expressing fly heads to hybridize a high-resolution, whole-genome tiling array. The analysis of the signal, normalized by the input DNA, resulted in clusters of positive binding peaks (Fig. S3). We considered genes to be associated with p53 if they had binding peaks in the promoter region, defined as 2 kb upstream and 1 kb downstream of the annotated transcriptional start site. Examination of the ChIP-chip data revealed that p53 binds the promoter region of ∼1,500 genes in tau-transgenic flies, of which ∼550 showed significant enrichment in tau-expressing neurons compared with controls [false discovery rate (FDR) < 0.01; Fig. 2*B*]. To provide a comprehensive picture of p53 transcriptional function in postmitotic neurons, we included the entire list of 1,500 genes bound in tau-transgenic animals in our subsequent analyses (Dataset S1).

An important physiological role for the p53 targets we identified here is consistent with our previous recovery of *th*, *par-1*, *Tao*, *cdi*, *chb*, *aop*, *dally*, and *Fmr1* in an unbiased forward genetic screen for modifiers of tau neurotoxicity (19). Each of these genes was bound by p53 in the present ChIP-chip experiment. Similarly, a number of the genes that we have previously shown to control tau neurotoxicity in pathway-focused studies are also p53 targets: *hep*, *Act5C*, *Drp1*, *sqh*, *WASp*, *zip*, *PHGPx*, *CycD*, and *eIF-4E* (5, 6, 20–22). In addition, the list of genes bound by p53 in our ChIP-chip experiment includes cell-cycle regulators (*p53*, *Gadd45*, *CycD, Cdt1*, and *Msh2*). Recovery of these cell-cycle genes is consistent with genetic evidence supporting a neuroprotective, rather than proapoptotic, role for the DNA damage checkpoint in postmitotic neurons (5, 6). Of note, vertebrate homologs of *p53*, *Gadd45*, *CycD*, *Cdt1*, and *Msh2* are known p53 targets, supporting conservation of p53 binding and function from flies to mammals (18, 23, 24).

We next determined whether particular cellular pathways were enriched for p53 binding. We used the list of p53 target genes

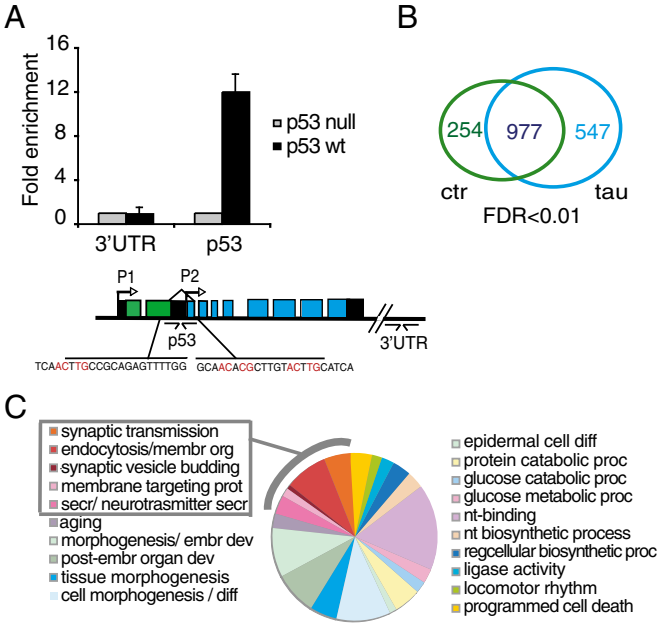

Fig. 2. Genome-wide p53 ChIP-chip analysis in *Drosophila* neurons. (*A*, *Upper*) ChIP assay showing that p53 binds its own gene promoter around the p53-responsive element but not the 3′ UTR, an unrelated region. Fold enrichment is expressed relative to negative control (*p53*-null flies). Values have been normalized to the total DNA and represent mean ± SEM. The graph is representative of one of the three independent triplicate assays. Flies are 10-d-old. (*Lower*) Schematic representation of the *Drosophila* p53 gene and the possible p53-responsive elements identified in the P2 promoter region. (*B*) Venn diagram of the ChIP-chip assay showing the number of genes bound by p53 in the control and tau-expressing animals. FDR < 0.01. (*C*) Pie chart representing the top 20 enriched pathways in the ChIP-chip analysis. The gray box indicates genes involved in synaptic function. See also Fig. S1 and Datasets S1–S3.

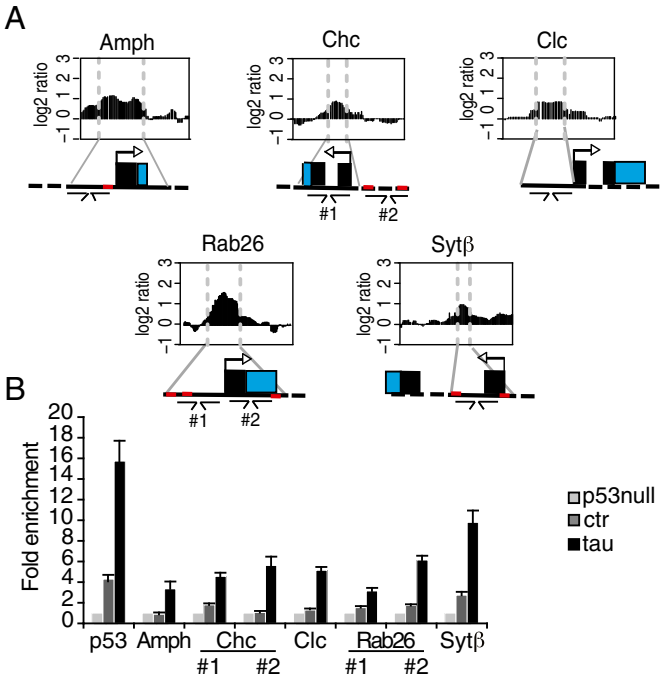

Fig. 3. Validation of genome-wide ChIP-chip results via traditional ChIP. (*A*) Diagram of the ChIP-chip binding peaks relative to the synaptic genes studied. The black boxes indicate the UTR, and the blue boxes indicate the first exon. The red lines represent p53 putative responsive elements (Fig. S2). (*B*) ChIP assay for p53 showing that p53 protein binds the selected synaptic genes. Fold enrichment is expressed relative to negative control (*p53*-null flies). Values have been normalized to the total DNA and represent mean ± SEM. The graph is representative of one of the three independent triplicate assays. Flies are 10-d-old.

bound in tau-transgenic flies, performed a Gene Ontology analysis (25, 26), and compared the results with all genes in the *Drosophila* genome. The two most significantly enriched pathways in our assay were the synaptic transmission and endocytosis pathways (Datasets S2 and S3). The top 20 most highly statistically enriched pathways are listed in Fig. 2*C*, along with a representation of the total number of genes in each of the pathways in the pie chart.

Genetic Validation of a p53-Dependent Pathway Involved in Neuroprotection. To validate functionally the top pathway recovered in our ChIP-chip experiment, we chose five genes: amphiphysin (*Amph*), clathrin light chain (*Clc*), clathrin heavy chain (*Chc*), RAS oncogene family (Ras-related protein Rab26), and synaptotagmin β (*Sytβ*). These genes have well-conserved human homologs and define aspects of both exocytosis and endocytosis. Further, the human homolog of *Amph*, bridging integrator 1 (*BIN1*), is linked genetically to Alzheimer's disease in patients (27–30). Because of the resolution limit of the ChIP-chip array, we could not pinpoint the precise position for p53-binding sites in the promoters of the selected genes. We therefore performed additional analysis to locate p53-binding sites. We searched for possible binding sites within the DNA sequences of the binding peaks derived from the ChIP-chip assay using the MatInspector software (31). In our in silico analysis, we considered the whole promoter region flanking the peaks (Fig. 3*A*). The MatInspector analytical software inspects for p53 half or full binding sites by using the vertebrate p53 consensus sequence. We tested the selected genes, and all but *Clc* have putative p53-binding sites within their promoter (Fig. 3*A* and Fig. S4).

We next confirmed the ChIP-chip and in silico analyses by performing traditional ChIP assays. We designed primers to amplify the genomic sequence corresponding to the ChIP-chip binding peaks and containing the p53-responsive elements (Fig. 3*A* and Fig. S4). For binding peaks that covered the genomic region >600–700 bp, we used multiple primers to study p53-binding activity for the entire region (*Chc* and *Rab26*) (Fig. 3*A*). We then analyzed chromatin from aged control and tau-transgenic flies and confirmed that p53 binds the promoters of the selected candidate genes (Fig. 3*B*). Because the physical association of p53 with the promoter region of the selected genes does not address directly induction of transcription, we performed an additional set of ChIP experiments using the H3K4me3 antibody. The H3K4me3 histone modification is associated with open and transcribed chromatin (32). All of the synaptic-function–related gene promoters bound by p53 also showed H3K4me3 histone modification (Fig. S5). Together, these data suggest that p53 binds to and promotes the transcription of genes involved in synaptic function.

We then validated the functional role of these novel p53 target genes in tau neurotoxicity using genetics. We found that reducing the expression of each of our five target genes significantly enhanced tau neurotoxicity as determined by TUNEL staining (Fig. 4*A*). In addition, we found a concomitant increase in PCNA when we reduced modifier genes, consistent with an effect upstream of cell-cycle activation (Fig. 4*B*). Increasing the levels of one candidate, *Rab26*, produced a trend toward suppression of neurotoxicity and cell-cycle reactivation (Fig. 4 *A* and *B*). In our genetic studies, we obtained significant results with at least two independent genetic reagents to confirm our results. In addition, we ensured that reduction of synaptic function genes did not cause toxicity in the absence of tau (Fig. 4 *A* and *B*) and that modifiers did not act simply by altering the levels of tau. We also

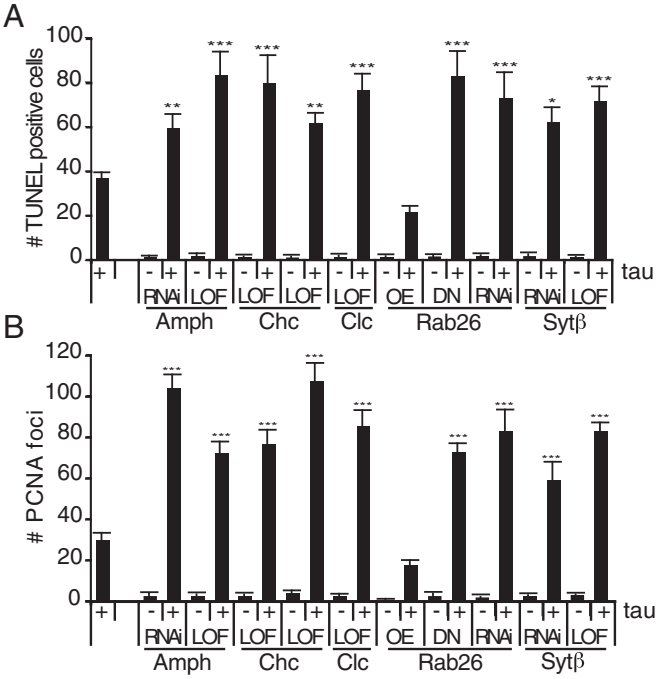

Fig. 4. Genetic manipulation of synaptic genes modifies tau neurotoxicity. (*A*) Synaptic genes modify apoptosis, indicated by TUNEL-positive neurons, in the brains of flies expressing tau. (*B*) Synaptic genes modify cell-cycle reactivation, indicated by PCNA-positive foci, in tau-transgenic flies. Values represent mean ± SEM ($n \geq 6$ per genotype). Asterisks indicate significant differences compared with control by ANOVA with Student–Newman–Keuls post hoc test for multiple comparisons. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. control, *elav-GAL4/+*; LOF, loss of function; OE, overexpression; DN, dominant negative.

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observed that reducing the expression of genes involved in synaptic function significantly worsened the locomotion behavior phenotype characteristic of tau-transgenic flies (Fig. S6). Conversely, increasing expression of Rab26 rescued locomotor function (Fig. S6). To evaluate further the specificity of the genetic modifications we observed, we tested our modifiers in a well-characterized *Drosophila* model of spinocerebellar ataxia type 3 (SCA3; Machado–Joseph disease) (33). We found no modification of mutant ataxin 3 toxicity when we altered expression of modifier genes involved in synaptic function (Fig. S7), suggesting that the modifiers we report do not show general nonspecific toxicity. Together, our biochemical, genetic, and behavioral data strongly suggest that p53 controls the transcription of a group of synaptic genes critical for protection from tau neurotoxicity.

To determine whether tau displays synaptic toxicity in our model, we assayed the levels of synaptic proteins, including presynaptic (synapsin, choline acetyltransferase, and syntaxin) and postsynaptic (discs large) markers in our tau-transgenic animals at baseline and in the absence of p53. We found significant reductions in each of these proteins in tau animals when p53 expression was removed, consistent with synaptic loss. Normal levels of the neuronal nuclear marker (elav) at the same time point suggest that loss of synapses was not simply secondary to neuronal loss (Fig. S8 *A* and *B*). In addition, we found evidence for abnormal synaptic proteostasis with accumulation of ubiquitin in synapses in tau-transgenic animals without p53 expression (Fig. S8*C*) and the presence of autophagy markers in the synapses of tau-transgenic flies (Fig. S8*D*). Together, these findings document synaptic pathology in tau-transgenic animals and correlate well with our ChIP-chip and genetic modifier data.

Synaptic Genes Are p53 Targets in Mammalian Neurons. Genome-wide ChIP-chip analysis followed by genetic validation in our simple genetic model of neurodegeneration represents a powerful approach to define the entire array of p53 family targets and perform functional analyses in vivo. However, the long-term goal of work is to understand the role of p53 in human disease states. We thus determined whether p53 controls the transcription of synaptic genes in mammalian neurons, using mouse primary cortical neurons. We transduced cortical neurons using lentivirus expressing two different shRNAs directed against the 3′ UTR or the ORF of p53. Assayed independently, both shRNAs were capable of reducing the levels of *p53* transcripts >60% compared with control (Fig. 5*A*, *Left*). Because we could obtain robust knockdown of p53 in primary neurons, we then assayed the expression of the mouse genes homologous to the p53-dependent, synaptic-function–related genes identified and validated in our *Drosophila* system. We observed a 60–80% reduction of all of the mouse genes studied following shRNA-mediated knockdown of p53 (Fig. 5*A*, *Right*).

To further assess the relevance of our model organism findings, we focused our attention on *BIN1*, the human homolog of *Amph*. We investigated *BIN1* in our human tissue studies because *BIN1* has recently been linked genetically to Alzheimer's disease. We identified putative p53-responsive elements in the promoter and in the first intron of the human *BIN1* gene (Fig. 5*B*). Then we performed ChIP from human postmortem frontal cortex using a p53-specific antibody and demonstrated, in three different individuals, that p53 binds the promoter and the first intron of the human *BIN1* gene (Fig. 5*C*).

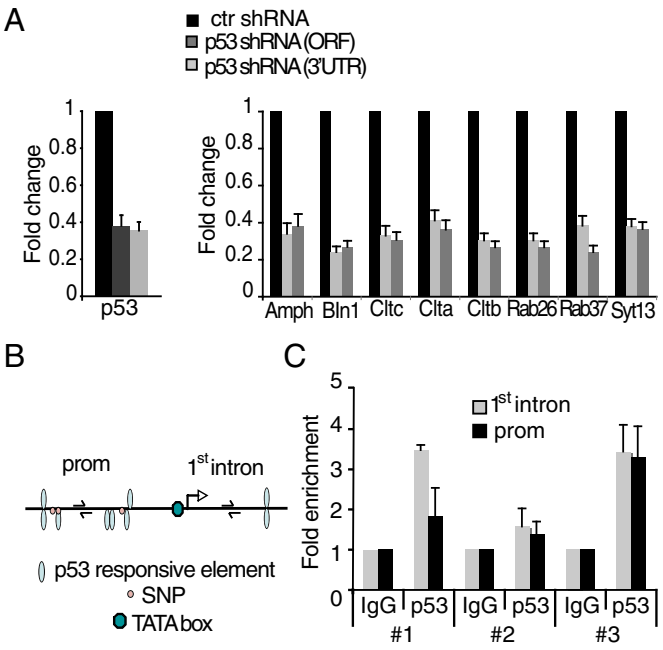

Fig. 5. p53 controls genes involved in synaptic function in mammals. (*A*, *Left*) Murine p53 transcript levels are reduced in primary cortical neurons transduced with either of two different lentivirus expressing *p53* shRNAs targeting the 3′ UTR or the ORF compared with cells transduced with the control shRNA. (*Right*) Synaptic gene transcript levels are reduced in primary cortical neurons transduced with lentivirus expressing p53 shRNAs compared with cells transduced with the control shRNA. In *A* and *B*, values, normalized to 18s RNA levels, represent mean ± SEM. The graph is representative of one of the three independent triplicate assays. (*B*) Schematic representing the human *BIN1* promoter and the putative p53-responsive elements identified upstream of the transcription start site and in the first intron region. (*C*) ChIP assay for p53 performed on human frontal cortex. Fold enrichment is expressed relative to IgG control. Values have been normalized to the total DNA and represent mean ± SEM. The graph shows results from three different patients, each analyzed in triplicate.

Discussion

Present (Fig. 1), and prior (5), studies from our laboratory have demonstrated that p53, best known as a critical tumor suppressor in oncogenesis, can play a neuroprotective role in postmitotic neurons. These data add substantially to the new roles for the p53 family of transcription factors that are emerging in postmitotic tissues (11, 12, 34). Our findings may have particular relevance in the context of neurological disorders because increased expression of p53 has been observed in adult neurons in a number of disease states (35, 36). The function of p53 in these postmitotic neurons has not been well defined experimentally. Alzheimer's disease, the most common human tauopathy, is one human disease in which p53 expression in neurons has been well documented. To investigate the mechanistic basis of p53-mediated neuroprotection in tauopathies, we have here characterized the genome-wide binding profile of p53 in aging, postmitotic neurons subjected to tau toxicity in vivo. In these studies we have identified and validated previously unidentified targets of p53 in postmitotic neurons. Interestingly, a group of genes controlling synaptic function emerged as the top pathway regulated by p53. The finding that p53 controls the transcription of genes encoding key synaptic vesicle exocytosis and recycling proteins reveals a novel aspect of the p53 response to cellular damage and suggests a protective molecular mechanism against one of the first manifestations of neurodegeneration. Synaptic pathology, including dysfunction and degeneration, is one of the earliest pathologic abnormalities in Alzheimer's disease and in experimental models of Alzheimer's disease and related tauopathies (37–39). Recovery of synaptic genes in our ChIP-chip experiment raises the possibility that, in response to DNA damage, p53 may protect postmitotic neurons from dysfunction and degeneration not only by preventing inappropriate reactivation of the cell cycle, but also by counteracting synaptic injury.

Importantly, we have here used the powerful genetics available in our model system to demonstrate that the top pathway identified in our profiling experiments, synaptic function, is fundamental for maintenance of postmitotic neurons subjected

to neurodegenerative stress during aging. Because our ultimate goal is to understand the pathways controlling neurodegeneration in human tauopathies, we have further demonstrated that p53 controls the expression of the same synaptic-function genes in murine cortical neurons. We additionally show that one specific human gene in the pathway studied, *BIN1*, is additionally controlled by p53 in the human brain. Thus, *BIN1/Amph* is a conserved p53 target from *Drosophila* to humans. These findings strongly suggest that the genes we have identified as previously unidentified targets of p53 in aging *Drosophila* neurons will serve as a valuable resource for functional analysis of the role of the p53 family in mammalian postmitotic neurons and in neurodegeneration.

Remarkably, six of the susceptibility genes so far recovered from genome-wide association studies in Alzheimer's disease patients are p53 targets in neurons in our study: *BIN1/Amph*, *PICALM/lap*, *CR1/CG10186*, *MEF2C/Mef2*, *SLC24A4/CG1090*, and *FERMT2/Fit1* (40, 41). p53 may therefore play a critical role in regulating genes involved in Alzheimer's disease pathogenesis, correlating with the known up-regulation of p53 in the disorder (35, 36). However, rather than promoting cell death, our data suggest that p53 may instead be part of an ancient and conserved protective stress response that acts in disease states to protect neurons and maintain critical neuronal systems, including, as we demonstrate here, synaptic function. Together, our findings may thus define pathways and molecules of potential therapeutic value in Alzheimer's disease and related neurodegenerative disorders.

Materials and Methods

Fly Stocks and Genetic Crosses. All fly crosses and experiments were performed at 25 °C. Tau was expressed panneuronally by using the *elav-GAL4* driver and the *UAS-tau^{R406W}* line (7). The *elav-GAL4* driver, *Amph^{EY09339}*, *Clc^{DG23206}*, *Chc^{1}*, *Chc^{4}*, *UAS-Rab26*, *UAS-Rab26^{T204N}*, *Sytβ^{DG10711}*, *UAS-Sytß RNAi JF02593*, and *p53^{5A-1-4}* were obtained from the Bloomington *Drosophila* Stock Center. The *Amph^{EY09339}* element is inserted in the inactivating orientation and is used as a loss-of-function mutation. *UAS-Rab26 RNAi* (line no. 43,730) and *UAS-Amph RNAi* (line no. 7,190) transgenic RNAi lines were obtained from the Vienna *Drosophila* RNAi Center. *p53$^{11-1B-1}$* and was kindly provided by Michael Brodsky, Program in Molecular Medicine, University of Massachusetts Medical School, Worcester MA. The presence of the *p53$^{11-1B-1}$* mutant allele in relevant stocks was confirmed by using PCR on genomic DNA (Fig. 1*A*, *Lower*) (42). Nancy Bonini, Department of Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, provided flies for expression of mutant ataxin 3. The *UAS-GFP-Atg8a* and *UAS-GFP-ref(2)P* reporters were obtained from T. Neufeld, Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis (43, 44). Complete genotypes can be found in *SI Text*.

Western Blot Analysis. Heads from adult 10-d-old flies were homogenized in Laemmli buffer, resolved by SDS/PAGE, and subject to immunoblotting analysis. Primary antibodies used were as follows: polyclonal anti-human tau (Dako; 1:10^{6}); anti-actin (Sigma-Aldrich; 1:20,000). From Developmental Studies Hybridoma Bank (DSHB), they were anti-Syn, anti-Cha, anti-discs large (4F3) (1:5,000); anti-Syx, anti-actin (JLA20) (1:1,000); and rat anti-elav (1:100). Before immunoblotting, nitrocellulose membranes were boiled (9 min in PBS) for antigen retrieval. HRP-conjugated secondary antibodies were used (1:50,000), and signal detection was performed with chemiluminescence (ChemiGlow West; Cell Biosciences). Western blots were repeated at least three times.

Histology and Immunohistochemistry. Adult flies were fixed in formalin at 10 d of age and embedded in paraffin. Serial frontal 4-μm sections including the entire brain were prepared. Antigen retrieval was performed by microwaving in sodium citrate buffer. Immunostaining was performed with immunofluorescence or an avidin–biotin–peroxidase complex method followed by detection with diaminobenzidine. The number of PCNA-positive foci was counted in the entire brain (anti-PCNA; Biomeda concentration). Neuronal apoptosis was detected with the TUNEL assay by using a commercially available kit (TdT FragEl; Oncogene). Primary antibodies used: anti-GFP (NeuroMab, N86/8; Invitrogen A-11122; 1:100); Csp (DSHB; 6D6; 1:20); UBQ (Dako; Z0458; 1:200); and PCNA (Biomeda; 1:500). To evaluate neuronal loss following expression of mutant ataxin 3, the number of neurons in a defined, ~250-μm^2 area of Kenyon cells was counted (45).

Locomotor Behavior. Flies were collected in fresh vials on the day of eclosion and aged for 24 h. Flies were then placed without anesthesia over a gridded surface, and the number of lines crossed in 30 s was counted. Flies were tapped gently to the same initial starting position at the beginning of each experiment. Data are presented as the number of centimeters walked in 30 s.

ChIP. For ChIP performed on *Drosophila* tissue, heads were selected through a sieve from 200 mg of frozen 10-d-old flies. Fly heads were fixed with 1.8% (vol/vol) formaldehyde, washed twice in ice-cold PBS, resuspended in radioimmunoprecipitation assay (RIPA) buffer plus protease inhibitor, and disrupted by sonication. Chromatin was sheared into lengths of 400–1,000 bp. A total of 1/100 of the clarified sample was stored as input, and the remaining was incubated with 2 μg of anti-p53 (d-200; Santa Cruz). We followed Millipore ChIP Assay directions for the next steps. DNA was resuspended in water and analyzed by quantitative PCR.

For ChIP performed on *Drosophila rpr* and *p53* genes, *w^{1118}* embryos were collected ~6 h after egg laying and exposed to 4,000 rad of gamma-irradiation. Embryos were allowed to recover at 25 °C for 1.5 h (18), dechorinated for 2.5 min in 50% bleach, and washed in tap water. A total of 60 mg of embryos were used for each condition. Embryos were dounced in a 2-mL homogenizer and resuspended in 750 μL of ice-cold NEEM buffer. After 10 strokes, the homogenate was collected, cross-linked in 1% paraformaldehyde for 15 min at room temperature, resuspended in 500 μL of RIPA plus inhibitors, and treated as indicated above.

For ChIP performed on human tissue, autopsy specimens were obtained from Brigham and Women's Hospital from three patients without neurological disease, including two females and one male, ages 71, 74, and 80 y. Frontal cortex (250 mg) was fixed in 1% formaldehyde, washed in ice-cold nuclei extraction buffer (NEB), and resuspended in NEB and 0.1% Triton X-100 plus inhibitors of proteases. Tissue was then homogenized in a 5-mL dounce homogenizer 50 times on ice, layered over a sucrose cushion [1.8 M sucrose, 3 mM $Mg(Ac)_2$, 10 mM Tris·HCl, pH 8], and centrifuged for 2 h at 25,000 rpm at 4 °C (Beckman L-80, SW28 rotor). The nuclear pellet was resuspended, and nuclei were disrupted by sonication. Chromatin was sheared into fragments of 300–800 bp. Immunoprecipitation was performed by using 5 μg of anti-human p53 (BD Pharmingen). Complete buffer recipes and primer sequences for the RT-PCR experiments are provided in *SI Text*.

ChIP-chip. The ChIP-chip experiment was performed as for ChIP on *Drosophila* heads with the addition of a final whole-genome DNA amplification step, performed with a WGA2 kit (Sigma). A total of 6.5 mg of purified and amplified chromatin were then submitted to NimbleGen/Roche for the labeling step and the hybridization to a HD2 whole-genome tiling array. We performed two replicates for each ChIP-chip experiment and normalized the data as described (25). For each replicate, we set signal (i.e., log ratio) median to zero and rescaled signals so that their SD is the average SD of the replicates. To identify binding clusters, we took the mean of the replicates and applied running median smoothing with a window size of 500 bp along chromosomal locations. On the basis of the DNA fragment size, clusters consisting of at least 10 probes (~550 bp) above a threshold were counted as significant. For a FDR of 0.01, this threshold is approximately three times the average SD. Genes with p53 binding in their promoter regions (from −2 to 1 kb near the transcription start site) are regarded as p53 target genes.

Gene Ontology Analysis. To examine whether particular gene sets were enriched for p53 binding at their promoters, we selected p53 target genes from tau-transgenic animals and used DAVID for Gene Ontology analysis (26). To reduce the redundancy in gene set annotation, we chose functional annotation clustering, which groups similar annotations together. We used high classification stringency. Results are summarized in Fig. 2*C*; full results are presented in Dataset S2. Dataset S3 provides other terms, which are functionally related to the top cluster.

Lentiviral Constructs Expressing shRNA and Infection of Cortical Neurons. Two different murine p53 shRNAs (targeting the 3′ UTR or the ORF of p53) and a nonmammalian shRNA (TRCN0000304241, TRCN0000310844, SHC002; Sigma) were subcloned in a pLKO.3G vector (Addgene) by using the Nde/BamHI enzymes. Restriction enzyme analysis and DNA sequencing confirmed correct insertion and integrity of shRNAs. Lentiviruses were generated by cotransfecting for 48-h pLKO.3G-shRNAs and packaging vectors (VSVG, RSV-REV, and pMDL g/p RRE) into 293T cells using the MIRUS-Transit293 reagent. The supernatant containing viral particles was purified and concentrated (Ultracel-30K; Millipore) to obtain a final titer of 10^{8} IU/mL. The recombinant lentiviruses were used to transduce embryonic-day 14.5 (E14.5) cortical neurons on the day of plating, and the transgene expression was confirmed postinfection by GFP expression.

Primary Neuronal Cultures. E14.5 mouse lateral telencephalons from the CD1 mouse strain were dissected out, and the pia were removed in cold sterile HBSS (Thermo Scientific) and dissociated by the Papain Dissociation System (Worthington Biochem) via incubation of tissue in papain and DNase I at 37 °C for 1 h. Cells were triturated 10 times by using sterile pasture pipets, and dissociated cells were spun down at 1,000 × *g* for 5 min. Cells were plated on plates that were precoated via incubation with poly-L-ornithine (Gibco) overnight at 37 °C. Cells were plated at a density of 100 K/cm^2 and cultured in Neurobasal Medium supplemented with 0.6% glucose, B27, N2, 2 mM L-glutamine, and 100 U/mL penicillin/streptomycin mixture (Gibco).

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