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REGULAR ARTICLE

Study of tauopathies by comparing *Drosophila* and human *tau* in *Drosophila*

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Abstract The microtubule-binding protein tau has been investigated for its contribution to various neurodegenerative disorders. However, the findings from transgenic studies, using the same *tau* transgene, vary widely among different laboratories. Here, we have investigated the potential mechanisms underlying tauopathies by comparing *Drosophila* (*d-tau*) and human (*h-tau*) *tau* in a *Drosophila* model. Overexpression of a single copy of either *tau* isoform in the retina results in a similar rough eye phenotype. However, co-expression of *Par-1* with *d-tau* leads to lethality, whereas co-expression of *Par-1* with *h-tau* has little effect on the rough eye phenotype. We have found analogous results by comparing larval proteomes. Through genetic screening and proteomic analysis, we have identified some important potential modifiers and tau-associated proteins. These results suggest that the two *tau* genes differ significantly. This comparison between species-specific isoforms may help to clarify whether the homologous *tau* genes are conserved.

Keywords *Tau* · Tauopathy · Comparison · Conservation · *Drosophila*

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Introduction

The microtubule-associated protein tau was first described as a heat-stable protein essential for microtubule assembly (Weingarten et al. 1975). Tau belongs to a family of microtubule-associated proteins and is primarily expressed in neurons in which it plays a major regulatory role in the organization and integrity of the cytoskeletal network (Shahani and Brandt 2002). The relationship between *tau* and two neurodegenerative diseases, Alzheimer's disease (AD) and frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), has been established based on the presence of mutations in the *tau* gene and of tau-laden lesions ("tangles") in these disorders (Kosik et al. 1986; Grundke-Iqbal et al. 1986a, b; Baker et al. 1997; Hutton et al. 1998; Spillantini et al. 1998; Poorkaj et al. 1998; Yancopoulou and Spillantini 2003). However, despite an enormous research effort to determine the association

between *tau* and AD, no known mutations or even polymorphisms found in the *tau* gene are associated with sporadic AD (Roks et al. 1999; Russ et al. 2001; Delacourte and Buee 2000).

Various studies have successfully introduced *tau* overexpression in cell lines and animal models to explore the mechanisms underlying neuronal degeneration in human diseases. Several animal models have been developed to examine the effects of *tau* overexpression (Götz et al. 1995; Hall et al. 1997; Wittmann et al. 2001; Tomasiewicz et al. 2002; Delobel et al. 2002; Kraemer et al. 2003). For example, *Drosophila melanogaster* has been widely used to investigate *tau*-related mechanisms in vivo (Jackson et al. 2002; Doerflinger et al. 2003; Nishimura et al. 2004; Mershin et al. 2004; Khurana et al. 2006; Chee et al. 2006; Chau et al. 2006). However, a determination of whether the mechanisms and interactions of homologous *taus* are conserved across species is required. This is particularly important because of the considerable disagreement in the field of tauopathies regarding the precise role and function of *tau* in neurodegeneration (Spittaels et al. 1999; Probst et al. 2000; Spittaels et al. 2000; Jackson et al. 2002; Shulman and Feany 2003).

In the present study, we have compared *Drosophila* (*d-tau*) and human (*h-tau*) *tau* in a *Drosophila* model to examine whether the regulative mechanisms of the *tau* genes are conserved in humans and *Drosophila*. We have found that the two *tau* genes share approximately half of the same functions. Thus, by comparing *d-tau* and *h-tau* in the *Drosophila* model, identical aspects of the *tau* function might be deduced.

Materials and methods

Fly stocks and genetics

Stocks were cultured on a standard cornmeal/molasses medium (Guo et al. 1996) at 25°C (unless otherwise mentioned) and 60% humidity under a 12-h:12-h light/dark cycle. The line carrying *UAS-h tau* (III) was the gift of Prof. Mel Feany (Harvard Medical School, USA; Wittmann et al. 2001). The *UAS-d tau* (II) line was the gift of Prof. Efthimios M.C. Skoulakis (BSRC, Greece; Mershin et al. 2004). *pGMR-GAL4* (II and III), *elav-GAL4* and *UAS-P35* were gifts of Prof. Kejing Deng (Fudan University, China). *UAS-Par-1/TM3* and *UAS-Par-1 KN/TM3* were gifts of Prof. Bingwei Lu (Stanford University School of Medicine, USA; Nishimura et al. 2004). *UAS-mts* and *UAS-dn mts* were gifts of Prof. Suzanne Eaton (Max Planck Institute of Molecular Cell Biology and Genetics, Germany; Hannus et al. 2002). The following lines: *w*^{1118}*; P{GT1}CG9238*^{BG02516}, *y*^{1} *P{SUPor-P}CG3011*^{KG08318}, *w***; Pka-C1*^{DN}*/CyO, y*^{1}*w*^{1118}*; P{UAS-arm.Exel}2, y*^{1}*; P{SUPor-P}CG9953*^{KG09912} *ry*^{506}, *y*^{1} *w*^{67c23}*; P{EPgy2}Gclm*^{EY13184}, *y*^{1} *w*^{1118}*; P{UAS-arm.Exel}3/TM3, Sb*^{1} *Ser*^{1}, *y*^{1} *w*^{67c23}*; P{SUPor-P}CG8889*^{KG05213}, *w*^{1118}*; P{UAS-sgg.S9A}MB14, y*^{1} *w***; Cam*^{n339}*/CyO, y*$^{+}$*; P{UAS-Cam.B34Q}3, w*^{1118}*; P{EP}tau*^{EP3203}, and *w*^{1118}*; PBac{PB}tau*^{c05068} were obtained from the Bloomington *Drosophila* Stock Center (Ind., USA). Double transgenes expressing *tau* and *pGRM-GAL4* were generated with the double balancer line *Adv/CyO; Sb/TM6B* according to standard genetic procedure (Li et al. 2004). The modifiers of the *tau*-induced rough eye phenotype were selected on the basis of their ability to modify the phenotype of *UAS-d&h tau/pGMR-GAL4* flies. Candidate modifiers were tested for their ability to modify the *UAS-d&h tau/pGMR-GAL4* animals (Shulman and Feany 2003).

Analysis of eye discs

Third instar larvae were dissected in *Drosophila* Ringer's solution (182 mM KCl, 46 mM NaCl, 3 mM $CaCl_2.2H_2O$, 10 mM TRIS-HCl, pH 7.2), and the separated eye-antenna discs were incubated for 5 min in a 1.6×10^{-6} M solution of acridine orange. Eye discs were rinsed in *Drosophila* Ringer's solution and photographed immediately by using a Nikon fluorescent microscope with 488-nm excitation light (Sullivan et al. 2000).

Scanning electron microscopy

For scanning electron microscopy, fly heads were detached carefully under the microscope to leave the eyes intact, fixed overnight in 2.5% glutaraldehyde, dehydrated in ethanol (once in 30%, 50%, 70%, 90%, and 95% ethanol, and then twice in pure ethanol, 10 min each), dried under a vacuum, attached to stubs, and analyzed by using a Hitachi scanning electron microscope (Sullivan et al. 2000). The solutions used above were prepared in phosphate-buffered saline (2.7 mM KCl, 4.3 mM $Na_2HPO_4.12H_2O$, 1.8 mM KH_2PO_4 and 137 mM NaCl).

Specimen preparation

Third instar larvae were collected, rinsed in 95% ethanol, which was allowed to evaporated in air, and homogenized in acetone (10% trichloroacetic acid, 0.07% β-mercaptoethanol). The extracts were cooled at −18°C for 4 h and then centrifuged at 22,000*g* at 4°C for 15 min. Each pellet was re-suspended in 1 ml acetone precooled at −18°C and re-centrifuged at 22,000*g* at 4°C for 20 min. The supernatants were discarded, and the pellets were rinsed two more times. After air desiccation, the pellets were re-dissolved in lysis buffer consisting of 8 M urea, 4% CHAPS, 40 mM dithiothreitole (DTT), 0.8% IPG buffer. A

protease inhibitor cocktail (Roche Applied Science, Germany) was added to this pellet mixture. The extracts were then centrifuged at 27,000*g* at 4°C for 1 h. Protein concentration was measured by the Bradford method, and the samples were stored at −78°C until use.

Two-dimensional electrophoresis and image analysis

Two-dimensional electrophoresis (2-DE) and image analysis were performed according to the methods described by Amersham Biosciences and Li et al. (2005). Briefly, the first dimension of IPG-DALT2-DE (Proteomics Platform, Institute of Biophysics, CAS, China) was run on an IPGphore isoelectric focusing (IEF) system (Amersham Biosciences). Total protein (200 μg) was mixed with rehydration solution (8 M urea, 2% CHAPS, 40 mM DTT, 0.8% IPG buffer, 0.002% bromophenol blue) and applied to IPG dry strips (pH 3–10 linear, 11 cm). After rehydration for 16 h, IEF was conducted according to standard procedures. Following IEF separation, the gel strips were equilibrated twice for 15 min in equilibration buffer (40 mM TRIS-HCl pH 8.0, 6 M urea, 30% glycerol, 2% SDS, 0.002% bromophenol blue). Of note, DTT (1%) was added to the first equilibration buffer, whereas in the second equilibration buffer, DTT was replaced with 4% iodinacetamide (IAA). SDS-polyacrylamide gel electrophoresis was carried out on a SE 600 system (Amersham Biosciences). Bands were visualized by silver staining. The silver-stained 2-D gels were scanned by using a Magic Scanner (Amersham Biosciences). Spot detection, quantification, and matching were performed by using PDQuest software (Amersham Biosciences). To confirm the spots that were detected, we visually compared the gels and did not further characterize random spots.

In-gel digestion

For mass spectrometry (MS) fingerprinting, gel slices were cut out of the silver-stained gels, rinsed in deionized water twice for 10 min, destained with 100 mM $K_3Fe(CN)_6$ and 30 mM $Na_2S_2O_3$ (V/V=1:1), dehydrated with acetonitrile until the slices turned white, and then dried completely by centrifugal desiccation. The dried gel slices were rehydrated in 10 mM DTT at 56°C for 1 h. After this solution had been removed, 55 mM IAA was added, and the slices were left in the dark for 45 min, whereafter the IAA was discarded, and the slices were rinsed stepwised with NH_4HCO_3 and 50% and 100% acetonitrile (ACN). The slices were dried again for 5 min and digested with 0.01 μg/μl trypsin at 4°C for 30 min. The slices were subsequently incubated at 37°C overnight in 25 mM NH_4HCO_3. Digestion was stopped with 2% trifluoroacetic acid (TFA). The digestion buffer was transferred to a new tube, and the gel slices were extracted with 60% ACN. The extracts were pooled and dried completely by centrifugal desiccation. Polypeptides were extracted with 0.1% TFA.

Protein identification

Each sample was identified by high performance liquid chromatography tandem MS (Thermo Finnigan, San Jose, Calif., USA) at the Institute of Zoology, CAS, China. Protein identification (Sequest software) criteria were based on Delta CN (>0.1) and Xcorr (one charge >1.5, two charges >2.0, three charges >2.5). Peptides were identified by using the National Center for Biotechnology Information (NCBI) protein database (http://www.ncbi.nlm.nih/).

Results

Overexpression of *d-tau* and *h-tau* in the retina

Overexpression of either the wild-type *d-tau* or *h-tau* in the *Drosophila* eye resulted in the rough eye phenotype (Fig. 1b,c) as described previously (Wittmann et al. 2001; Jackson et al. 2002; Chau et al. 2006). To determine whether

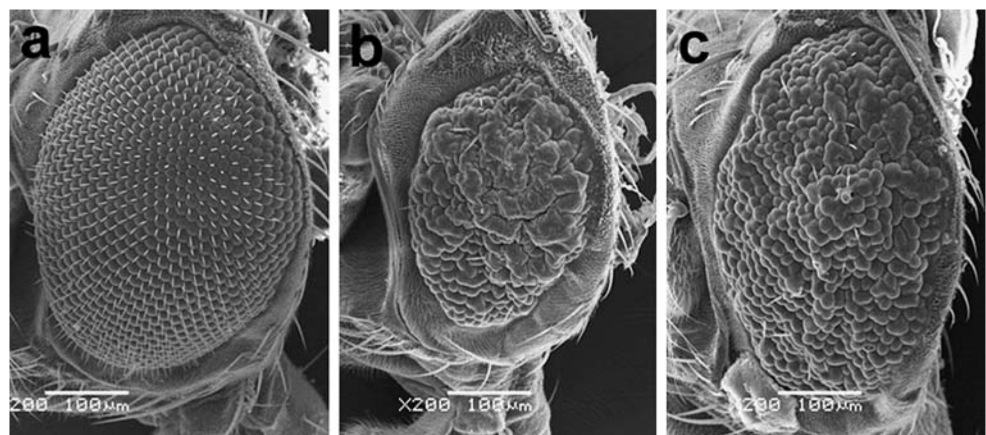

Fig. 1 Rough eye phenotype of *tau*-overexpressing lines. Scanning electron-microscopic images of fly eyes. Genotypes: (**a**) *pGMR-GAL4/+*, (**b**) *UAS-d tau/pGMR-GAL4* (*III*), (**c**) *UAS-h tau/ pGMR-GAL4* (*II*). The two transgenic lines show a similar rough eye phenotype. All flies were 1 day old

Table 1 Modifiers of the two *tau* genes (*None* no apparent effect)

Gene	Mammalian homolog/function	Line	Modification of *d-tau*	Modification of *h-tau*
par-1	MARK serine/threonine kinase	*w; UAS-Par-1/TM3*	Lethal	Enhancer
par-1 kn	MARK serine/threonine kinase	*w; UAS-Par-1 KN/TM3*	None	Suppressor
sgg.S9A	Glycogen synthase kinase 3	w^{1118}*; P{UAS-sgg.S9A}MB14*	Enhancer	None
mts	PP2A phosphatase subunit C	*w; UAS-mts*	Lethal	Lethal
dn mts	Dominant negative type of PP2A phosphatase subunit C	*w; UAS-dn mts*	Enhancer	Enhancer
Pka-$C1^{DN}$	Dominant negative form of cAMP-dependent protein kinase	*w*; Pka-*$C1^{DN}$*/CyO*	Suppressor	Suppressor
arm	β-Catenin	$y^{1}w^{1118}$*; P{UAS-arm.Exel}2*	Enhancer	Enhancer
arm	β-Catenin	$y^{1}w^{1118}$*; P{UAS-arm.Exel}3/TM3, Sb*^{1}*Ser*^{1}	None	Suppressor
P35	Pasadena 35	*w; UAS-P35*	Suppressor	Suppressor
CG9238	Protein phosphatase type 1 regulator	w^{1118}*; P{GT1}CG9238*^{BG02516}	Suppressor	None
CG3011	Glycine hydroxymethyltransferase	y^{1}*P{SUPor-P}CG3011*^{KG08318}	Enhancer	Suppressor

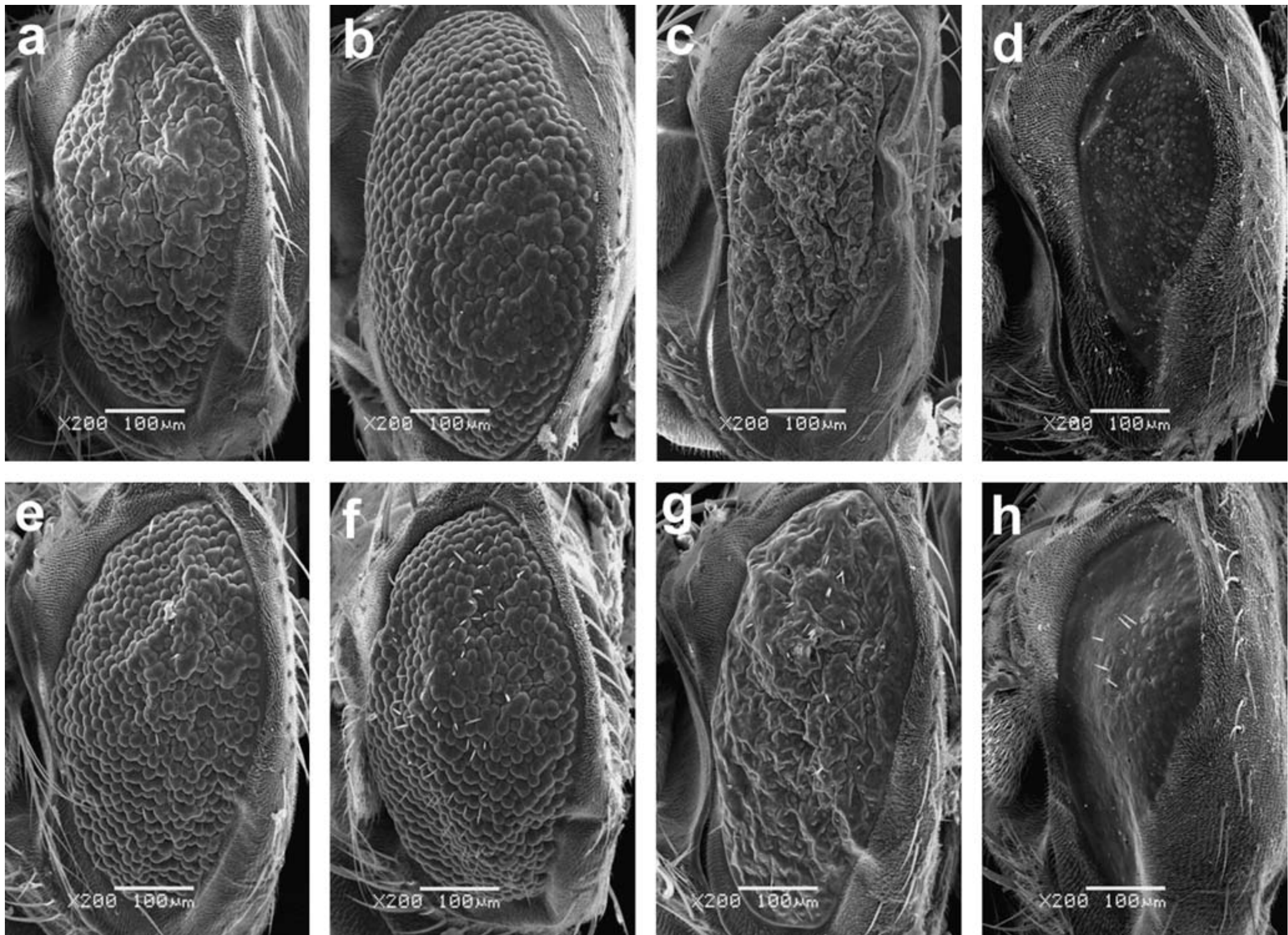

Fig. 2 Similar effects of gene modifiers on the phenotype of *tau*-overexpressing flies. Genotypes: (**a**) *Pka-C1*^{DN}*/UAS-d tau/pGMR-GAL4 (III)*, (**b**) *UAS-P35/UAS-d tau/pGMR-GAL4 (III)*, (**c**) *UAS-mts DN/UAS-d tau/pGMR-GAL4 (III)*, (**d**) *P{UAS-arm.Exel}2/UAS-d tau/pGMR-GAL4 (III)*, (**e**) *Pka-C1*^{DN}*/UAS-h tau/pGMR-GAL4 (II)*, (**f**) *UAS-P35/UAS-h tau/pGMR-GAL4 (II)*, (**g**) *UAS-mts DN/UAS-h tau/pGMR-GAL4 (II)*, (**h**) *P{UAS-arm.Exel}2/UAS-h tau/pGMR-GAL4 (II)*. The leading part of each genotype name indicates the line screened to enhance or suppress the rough eye phenotype

tau overexpression had caused cell death at an earlier stage of development, we dissected third instar larvae and stained the eye discs with acridine orange. We found more fluorescence in the eye discs of *tau*-overexpressing flies than that in the wild-type flies (not shown), indicating that *tau* overexpression had led to additional cell death. These results suggested that increased *tau* was toxic to cells.

Overexpression of *tau* genes on both alleles

Strikingly, we found that flies overexpressing two copies of *h-tau* (driver: *pGMR-GAL4*) generated with a double-balancer line all died before adulthood, whereas some flies with two copies of *d-tau* could survive under the same conditions, although the flies showed a more severe rough eye phenotype than did the flies overexpressing a single copy of *d-tau* (not shown). These results suggested that excessive *tau* was toxic to the flies, and that the influence of *tau* overexpression was dosage-dependent.

Interactions of *tau* with other genes in *Drosophila* eye

To determine whether the two homologous *tau* genes shared similar interactions with other genes in the *Drosophila* eye, we selected some well-known lines to screen the modifiers of *tau* (Robertson et al. 1993; Hay et al. 1994; Jackson et al. 2002; Hannus et al. 2002; Scherzer et al. 2003; Shulman and Feany 2003; Nishimura et al. 2004). As shown in Table 1, approximately half of the interactions of the two homologous *tau* genes with other genes in the eye were identical (Fig. 2), whereas the remaining evaluated interactions were divergent (Fig. 3). We also found two novel genes that appeared to regulate the rough eye phenotypes of the *tau*-overexpressing flies, including *CG3011* and *CG9953* (Table 1, Fig. 3) whose mRNAs were significantly up-regulated in *h-tau* transgenics (Shulman and Feany 2003). These results suggested that the molecular mechanisms of the homologous *tau* genes were conserved.

Perhaps the most intriguing finding was that co-expression of *Par-1* with *d-tau* led to lethality, whereas co-expression of *Par-1* with *h-tau* had little effect on the rough eye phenotype. This result differed from that previously obtained (Nishimura et al. 2004; Shulman and Feany 2003). Importantly, however, several modifications to the *tau* gene also differed between the two species (Table 1). Moreover, in contrast to a previous report (Jackson et al. 2002), *shaggy (S9A)* had no apparent interaction with *h-tau*, whereas it had an exacerbating effect on *d-tau*. These results demonstrated that differences in *tau* could lead to distinct effects in the same *Drosophila* model.

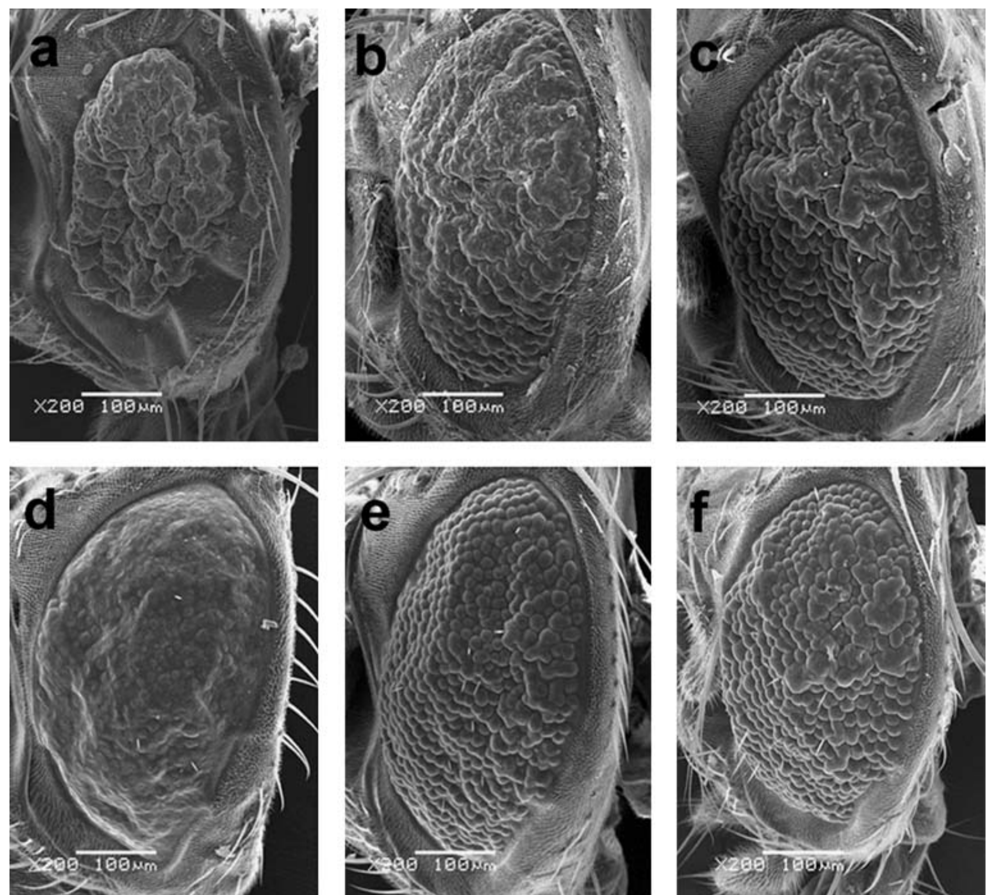

Fig. 3 Differential effects of gene modifiers on the phenotype of *tau*-overexpressing flies. Genotypes: (**a**) *P{UAS-sgg.S9A}MB14/UAS-d tau/pGMR-GAL4* (*III*), (**b**) y^1 *P{SUPor-P}* $CG3011^{KG08318}$*/UAS-d tau/pGMR-GAL4* (*III*), (**c**) *P{GT1}* $CG9238^{BG02516}$*/UAS-d tau/pGMR-GAL4* (*III*), (**d**) *UAS-Par-1/UAS-h tau/pGMR-GAL4 (II)*, (**e**) *P{UAS-arm.Exel}3/UAS-h tau/pGMR-GAL4 (II)*, (**f**) *UAS-Par-1 KN/UAS-h tau/pGMR-GAL4 (II)*. The reverse results are not shown, as no change was observed compared with the phenotypes of *UAS-d tau/pGMR-GAL4* (*III*) and *UAS-h tau/pGMR-GAL4 (II)* flies. The leading part of each genotype is the line screened to enhance or suppress the rough eye phenotype

Proteomes of larvae overexpressing *d-tau* and *h-tau*

Because the influence of *tau* expression may be more significant in the early stages of neurodegeneration, we further examined larvae for protein expression associated with *tau* at the late third instar. To study the protein expression patterns of the *tau*-overexpressing larvae (driver: *elav-GAL4*), we evaluated the total proteins by 2-DE (Fig. 4) across three independent experiments and observed little variability among the proteins found to be up-regulated. The protein expression patterns of the three transgenic lines closely resembled one another (not shown). We compared the gels and chose the differentially expressed protein spots (green and red circles, Fig. 4) whose expression levels varied by more than twice between the transgenic and the control animals. We evaluated these spots by mass spectrometry.

As expected, there were five proteins with differential expression between *d-tau* and *h-tau* compared with the control (Table 2). These same changed proteins, including CG9796-PA (a putative ortholog of gamma-interferon-inducible lysosomal thiol reductase precursor, blasted at http://www.dec2004.archive.ensembl.org/), CG13037-PA (mitochondrial ribosomal protein S34), CG7250-PA (Toll 6, transmembrane receptor protein Ser/Thr kinase), CG15093-PA (3-hydroxyisobutyrate dehydrogenase), and LP07910p (fat body protein 1), play important roles in protein metabolism or immune responses (Bertram et al. 2006). Moreover, *CG7250-PA* has a homologous gene, *TLR4*, which is known to be an AD-associated gene (ALZgene); *CG13037-PA* also has a homologous ALZgene *LOC439999* (Lemaitre et al. 1995). To our knowledge, this is the first report to associate these proteins with tau. Curiously, we did not identify d-tau and h-tau in the differentially expressed proteins, and this might have been attributable to low expression levels. Further research regarding the relationship between tau and various proteins may shed light on the early functional changes in tauopathies.

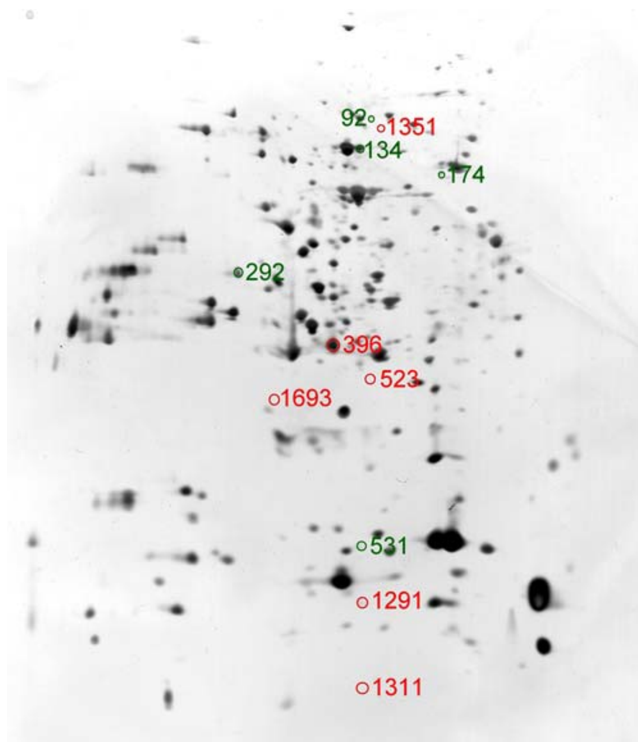

Fig. 4 The 2-DE pattern (11 cm) of whole proteins extracted from late third instar larva. The gel shown is a control. The *red-circled spots* indicate up-regulated proteins in the transgenic flies relative to the control (*elav-GAL4/+*), whereas the *green-circled spots* indicate down-regulated proteins in the transgenic animals relative to control

Discussion

In the present study, we have overexpressed *d-tau* and *h-tau* in a *Drosophila* model to determine whether the regulatory mechanisms of the two *taus* are conserved. We have found that the two *tau* genes have some functions in common, in addition to salient differences. Unfortunately, we have been unable to determine the conservation between the two *taus* from our primary data.

Modifiers of *tau*

We have selected some well-known *tau*-related genes, together with several additional potentially related genes, for screening (Robertson et al. 1993; Hay et al. 1994; Jackson et al. 2002; Hannus et al. 2002; Scherzer et al. 2003; Shulman and Feany 2003; Nishimura et al. 2004). Two novel gene modifiers have been identified (Table 1). Various other genes have been previously classified as *tau*-associated genes. We have also deduced those proteins that are tau-associated (Table 2) by proteomic analysis. As expected, the findings from the proteomic analysis correspond to those of the genetic screen, and both approaches suggest analogous functional conservation between *d-tau* and *h-tau*. Moreover, the differentially expressed proteins identified by proteomics might contribute to various tau mechanisms both under normal conditions and in neurodegeneration. Recently, puromycin-sensitive aminopeptidase has been identified as an inhibitor of tau-induced neurodegeneration by microarray analysis (Karsten et al. 2006). Taken with this finding, our results support the use of *Drosophila* models for research on *tau* and neurodegeneration.

Conservation between *d-tau* and *h-tau*

Our results indicate that the *tau* genes from humans and *Drosophila* share interactions with tau-associated proteins.

Table 2 Proteins with differential expression (>2-fold increase or decrease) between transgenic and control flies. The spots selected were identified with LC-MS/MS by using TurboSequest software (Thermo Finnigan). Comparisons were made between the control (*elav-GAL4/+*) and *d-tau*-overexpressing flies, and between the control (*elav-GAL4/+*) and *h-tau*-overexpressing flies (*SSP number* standard spot number, *% by mass* percent of amino acid homology between peptides found by mass spectrometry and the identified protein, *Functional classification* functional characters of the identified proteins)

SSP number	NCBI accession no.	kDa/pI	% by mass	Sequence tag	Protein identified	Functional classification
Up-regulated proteins in *d-tau*-overexpressing flies						
396	NP_650287	27.69/7.01	13.64	FITEQVYPAVKGELR AQVNLVGTICQYVSAPQPR	CG9796-PA	None
523	NP_724184	64.70/6.51	9.25	NDYFEMFAPK GCCNACEKPIVGQVITALGK DGFPYCEPDYHNLFSPR NFFERDGFPYCEPDYHNLFSPR	Paxillin CG31794-PF, isoform F	Focal adhesion
1693	NP_524104	21.61/9.54	5.20	KVEPVILPTK	Mitochondrial ribosomal protein S34 CG13037-PA	Protein biosynthesis
1351	NP_524081	170.16/6.02	0.72	LRLALPLPNGR	Toll-6 CG7250-PA	Transmembrane receptor protein Ser/Thr kinase
Down-regulated proteins in *d-tau*-overexpressing flies						
134	AAO24985	75.91/9.16	25.57	IFLGPAEDQQGR QVVQDNNIEQIDR SRQVLAQIGQIEQR IVDEQREQILGGYR QEQLQMR LSDPVVQYTLR LNQESIAQGQLIEEQQQLINNPR GQVGIMTIIR QEVIGQVLNQVNVNSLR VESLIADVLLGR RIEEHELDLSNLVEQQVQGIQQEIVGR EEHELDLSNLVEQQVQGIQQEIVGR	Fat body protein 1 LP07910p	Protein transporter
292	NP_725824	33.88/8.37	3.93	DYAGGFSSALITK	CG15093-PA, isoform A	Amino acid metabolism
531	AAO39536	93.12/6.36	4.12	KTLVLLGAHGVGR	RE09582p	Protein amino acid phosphorylation
Up-regulated proteins in *h-tau*-overexpressing flies						
396	NP_650287	27.69/7.01	13.64	FITEQVYPAVKGELR AQVNLVGTICQYVSAPQPR	CG9796-PA	None
1291	AAM50904	17.19/6.10	13.52	IHIQLAGPGVEVHESDEVHQK	LP06572p	None
1311	NP_729959	116.22/5.92	2.08	LLDEHQVYSVGRLEHVQQLR	Fat body protein 1 CG17285-PB, isoform B	Protein transporter
1351	NP_524081	170.16/6.02	0.72	LRLALPLPNGR	Toll-6 CG7250-PA	Transmembrane receptor protein Ser/Thr kinase
1693	NP_524104	21.61/9.54	5.20	KVEPVILPTK	Mitochondrial ribosomal protein S34 CG13037-PA	Protein biosynthesis
Down-regulated proteins in *h-tau*-overexpressing flies						
92	NP_724177	56.59/5.98	7.97	KVTTTASAPQLVQPVASSR IVEQPTQVTQTVPVQTAHYYQR	CG15825-PA, isoform A	None
134	AAO24985	75.91/9.16	25.57	IFLGPAEDQQGR QVVQDNNIEQIDR SRQVLAQIGQIEQR IVDEQRE QILGGYRQEQLQMR LSDPVVQYTLR LNQESIAQGQLIEEQQQLINNPR GQVGIMTIIR QEVIGQVLNQVNVNSLR VESLIADVLLGR RIEEHELDLSN LVEQQVQGIQQEIVGR EEHELDLSNLVEQQVQGIQQEIVGR	Fat body protein 1 LP07910p	Protein transporter
174	AAM11323	127.00/6.09	1.04	QLSWHLLRHK	SD09067p	None
292	NP_725824	33.88/8.37	3.93	DYAGGFSSALITK	CG15093-PA, isoform A	Amino acid metabolism

This is supported by the finding that the predicted *Drosophila* tau protein shares homology across species (Heidary and Fortini 2001). Our results agree with those of previous reports (Torroja et al. 1999; Spittaels et al. 2000; Wittmann et al. 2001; Jackson et al. 2002; Nishimura et al. 2004; Chau et al. 2006). The most significant finding regarding the homology between the two species is the rough eye phenotype that emerges following overexpression of either *d-tau* or *h-tau*, and our genetic screen is based on this identity. In addition, the introduction of two copies of either *tau* gene results in increased toxicity.

Further support emerges from the interactions between *tau* and other genes. For example, *P-35* prevents cell death (Hay et al. 1994) caused by *tau* overexpression, which suggests that both *d-tau* and *h-tau* play a role in apoptosis. Furthermore, the result from the up-regulation of Toll 6 in both types of *tau* transgenic flies suggests that *tau* may be involved in the immune response (Lemaitre et al. 1995). The common proteins with a differential expression compared with the control between *d-tau* and *h-tau* transgenic flies include half of the total proteins. Taken together, our findings suggest that *tau* may be functionally conserved between *Drosophila* and humans.

Differences between homologous taus

The differences in tau functions between *Drosophila* and humans may derive from the different structures of the two tau proteins (Heidary and Fortini 2001). We have found many differences in the interactions between various genes and the two *tau* genes, together with some novel findings (Jackson et al. 2002; Shulman and Feany 2003). Using proteomics, we estimate that approximately half of the interactions studied differ between *d-tau* and *h-tau*. Furthermore, previous experiments in mice (Spittaels et al. 1999, 2000; Probst et al. 2000) are consistent with the results presented here. Thus, different results can be obtained from different laboratories, even when using the same *tau* transgenic models. The sum of these findings suggests that, when various *tau* isoforms are expressed in *Drosophila*, the gene and the expressed product behave similarly but can also exhibit significant differences in mechanisms and interactions, underscoring the importance of genetic background.

Investigation of tauopathies by comparing homologous taus

The present results are consistent with our expectation that the two *tau* genes share some common modifiers and associated proteins. Our results further suggest the importance of ascertaining the conservation between the *tau* studied and the *tau* of the model system, in order to better compare studies across laboratories. A comparison of *d-tau* and *h-tau* in the *Drosophila* model in the present study helps to clarify the degree of homology, and the comparison also validates the views expressed in a previous review (Sang and Jackson 2005).

Some additional points should be mentioned with respect to the present work. Important functional domains may be identified by comparing functions and relative structures of the two *tau* genes and protein products. Such a comparison may help to eliminate, or at least identify the influence of, the genetic background in a model system. Trivial differences in breeding conditions, such as food composition, light levels, ambient noise, and housing climate, may lead to animals exhibiting different genetic expression patterns in different laboratories. As a result, variable genetic backgrounds might result in divergent results. In addition, the phenotype of *tau* overexpression depends greatly on the *tau* expression level; for example, *UAS-d &h tau/pGMR-GAL4* flies exhibit a rough eye phenotype, whereas *UAS-d &h tau/elav-GAL4* flies do not. Therefore, the different expression levels of *tau* in animals may model different types of tauopathies.

In conclusion, scrutiny of *d-tau* and *h-tau* together in an integrative study of *Drosophila* may help to clarify further the way in which aberrant *tau* changes can result in tauopathies.

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