TAUB INSTITUTE
for research on
ALZHEIMER'S DISEASE
and the aging brain

COLUMBIA UNIVERSITY
MEDICAL CENTER

Regulación de proteínas tau y neurodegeneración

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Department of Pathology & Cell Biology
Taub Institute for Research on Alzheimer's Disease and the Aging Brain
Columbia University Medical Center

Background: tau proteins

Proc. Nat. Acad. Sci. USA
Vol. 72, No. 5, pp. 1858–1862, May 1975

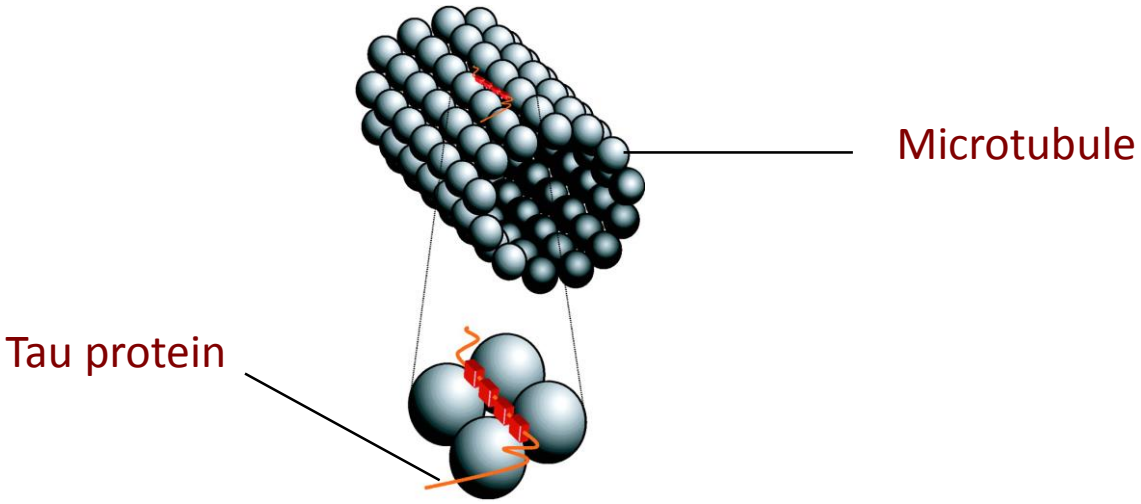
A Protein Factor Essential for Microtubule Assembly

(tau factor/tubulin/electron microscopy/phosphocellulose)

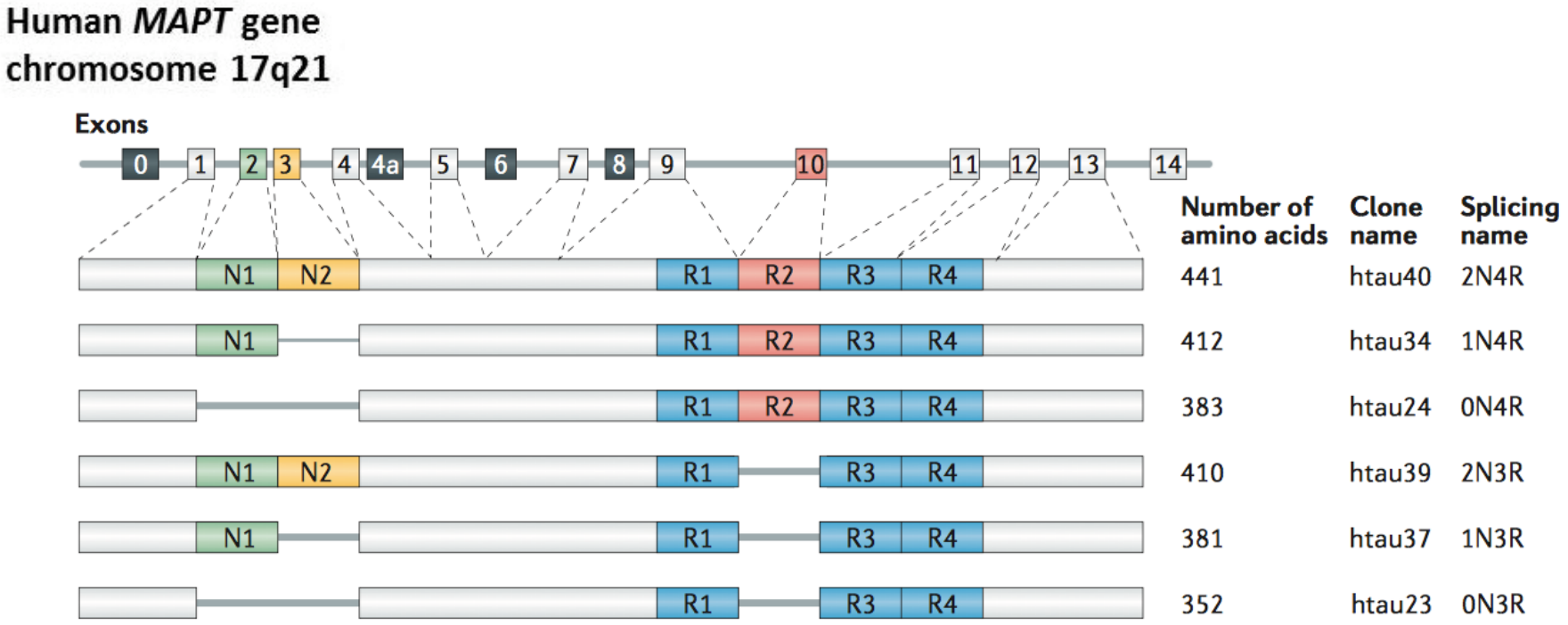
MURRAY D. WEINGARTEN, ARTHUR H. LOCKWOOD, SHU-YING HWO, AND MARC W. KIRSCHNER

Department of Biochemical Sciences, Moffett Laboratories, Princeton University, Princeton, New Jersey 08540

…….we propose to call this protein tau (T) for its ability to induce tubule formation.



Background: tau proteins



The human MAPT gene and the splice isoforms of tau in the human brain

Background: tau function

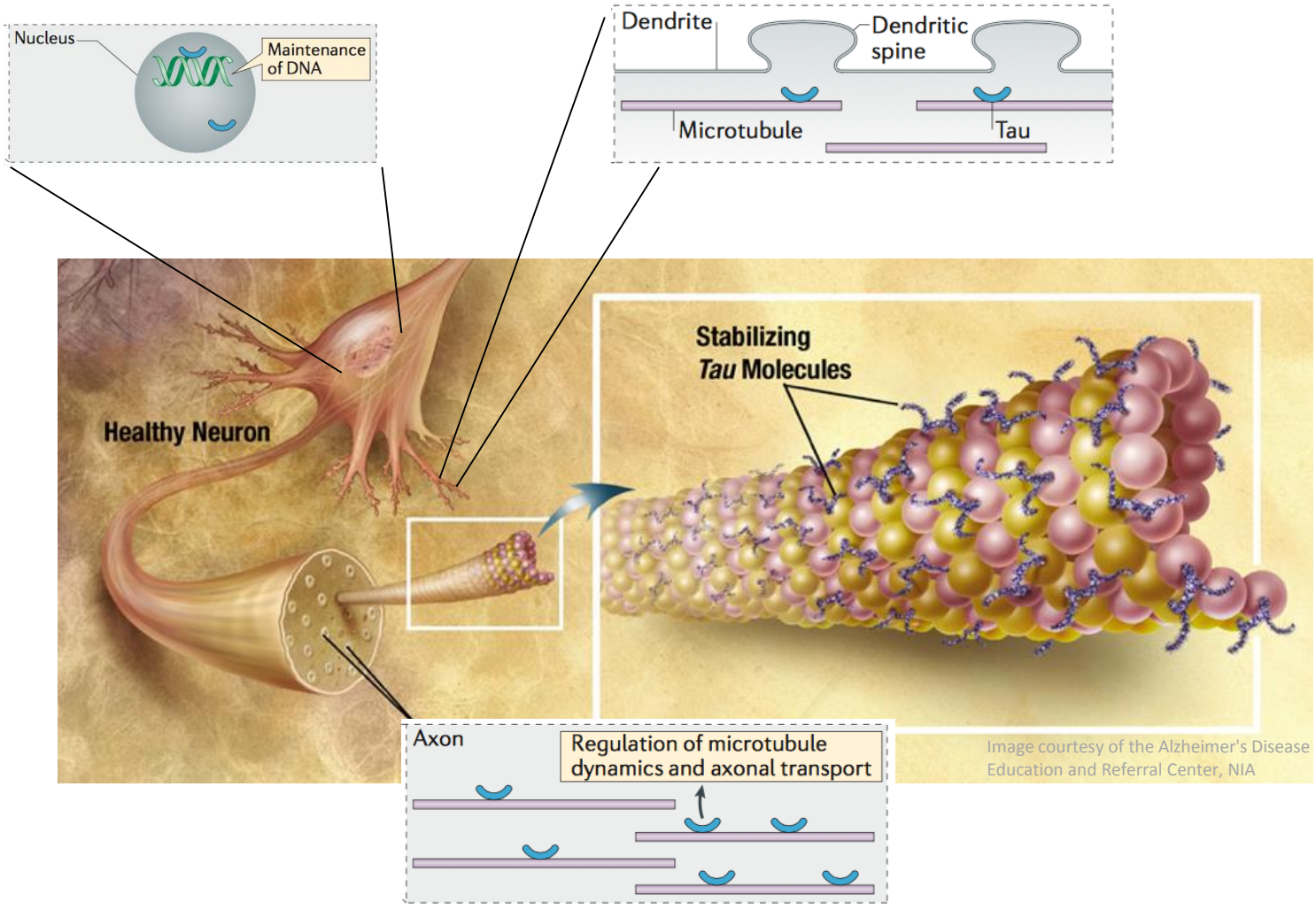


Image courtesy of the Alzheimer's Disease Education and Referral Center, NIA

Background: tau dysfunction, tauopathies

Aβ-associated

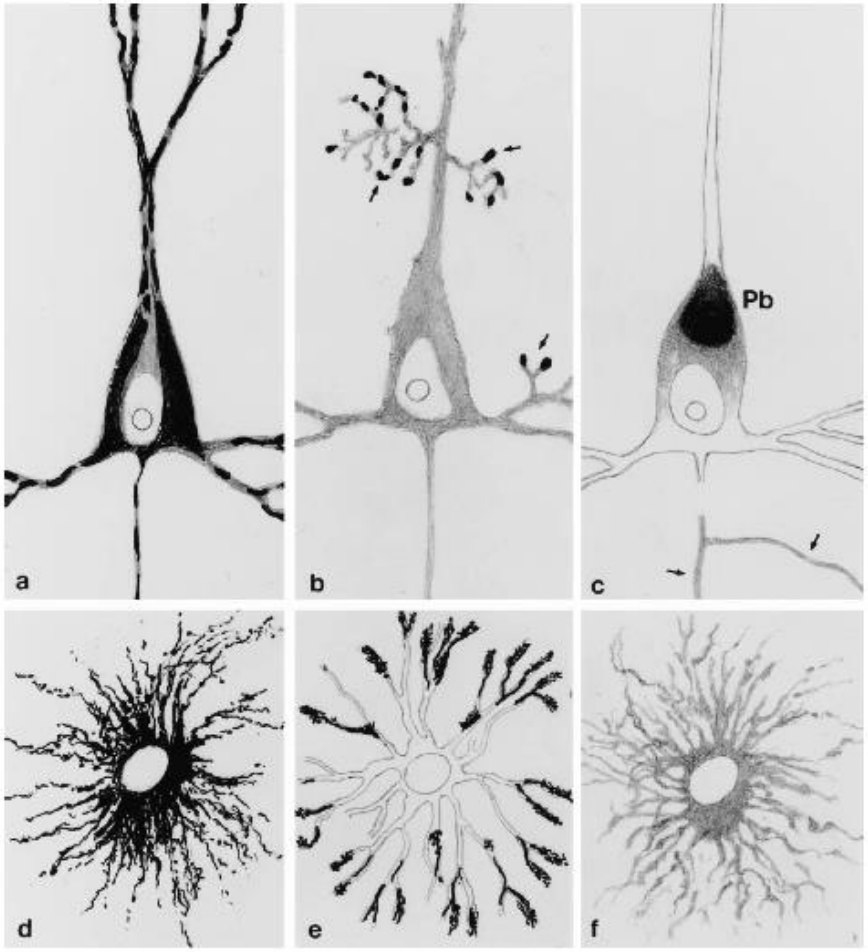
- Alzheimer Disease
- Down syndrome
- Familial British & Danish dementias

Predominantly tau

- Primary Age-related Tauopathy
- Chronic traumatic encephalopathy
- Progressive supranuclear palsy
- Corticobasal degeneration
- Pick disease
- Argyrophilic grain disease
- Lytico-Bodig disease (ALS/PDC of Guam)
- FTLD with *MAPT* mutation

Associated with other pathologies

- Postencephalitic parkinsonism
- Subacute sclerosing pan-encephalitis
- Myotonic dystrophy

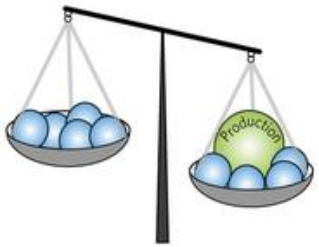


Neuropathology and Applied Neurobiology 24, 171-187

Clinical: cognitive impairment (dementia)/movement disorder
Neuropathological: wide spectrum of phenotypes
Biochemical: Differences in isoform composition

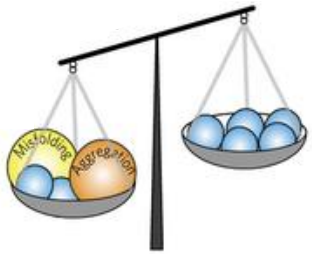
Background: Neurofibrillary degeneration

Tau protein production



→ Increased tau transcription?
→ Alterations in tau splicing?
→ Increased tau synthesis?

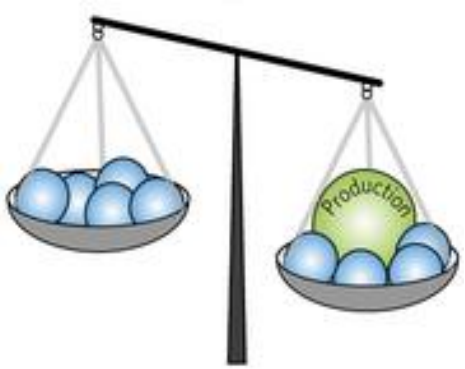
Post-translational modifications



→ Tau misfolding
→ Decreased protein degradation

Imbalanced state of tau proteostasis

Background: Neurofibrillary degeneration

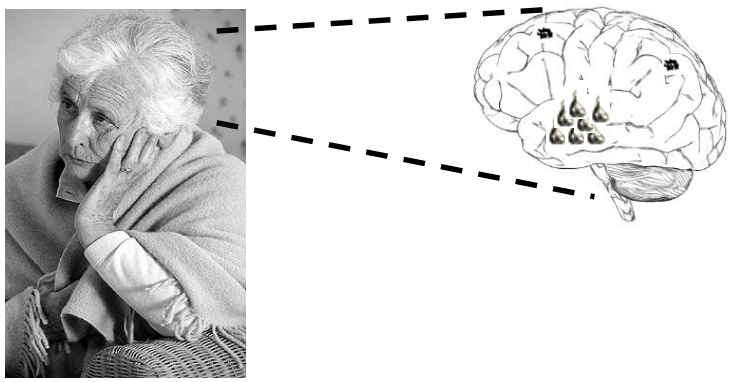


Increased tau protein production

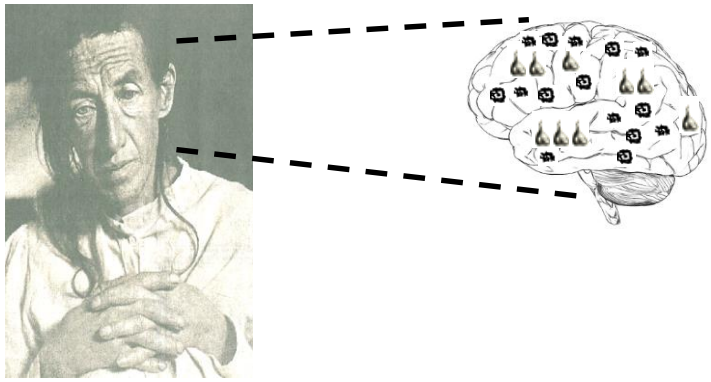
Aberrant transcriptional regulation?

Aberrant post-transcriptional regulation?

Background: Primary-age related tauopathy vs Alzheimer's disease



Primary-age related tauopathy (PART)



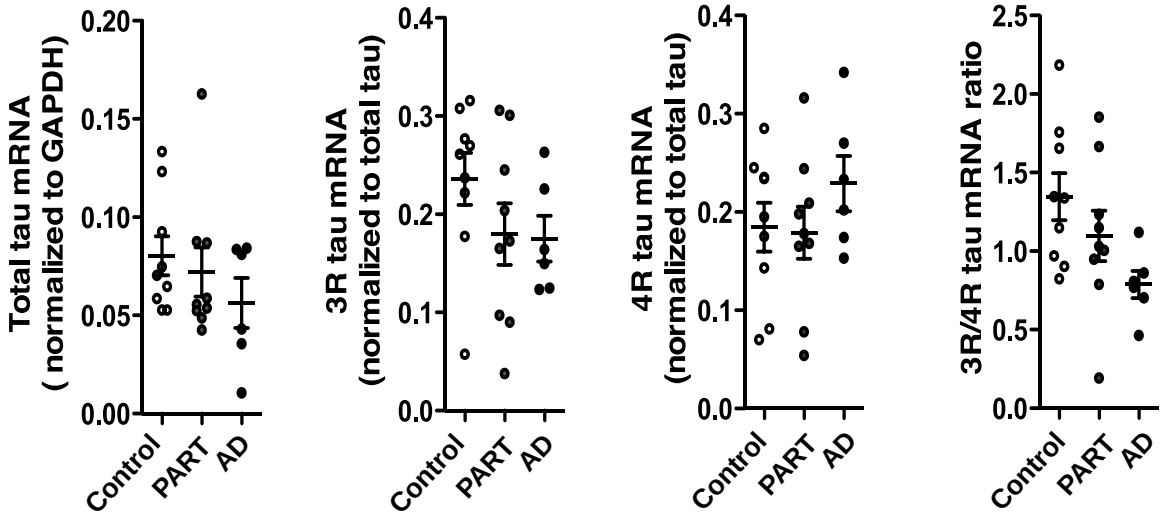
Alzheimer's disease (AD)

PART patients display Alzheimer-type tangles that are regionally, morphologically, ultrastructurally and biochemically similar to those in moderate-stage Alzheimer disease;

→ PART cases are 80+ years old
→ PART shares clinical features with AD including amnestic dementia.
→ But develop in the absence of amyloid-beta, soluble or otherwise
→ without a *MAPT* coding region mutation

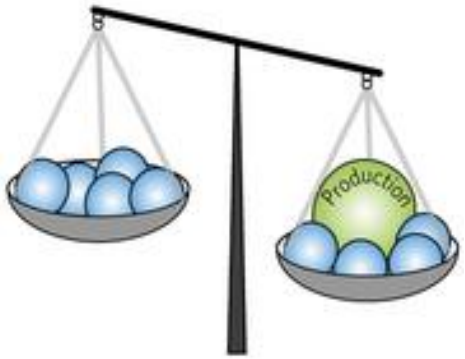
Background: Neurofibrillary degeneration

Aberrant transcriptional or splicing regulation in AD or PART?



QPCR analysis **does not** reveal significant differences in the levels or ratio of tau mRNA (i.e., total, 3R and 4R) among Primary age-related tauopathy (PART), Alzheimer Disease (AD) and controls.

Background: Neurofibrillary degeneration



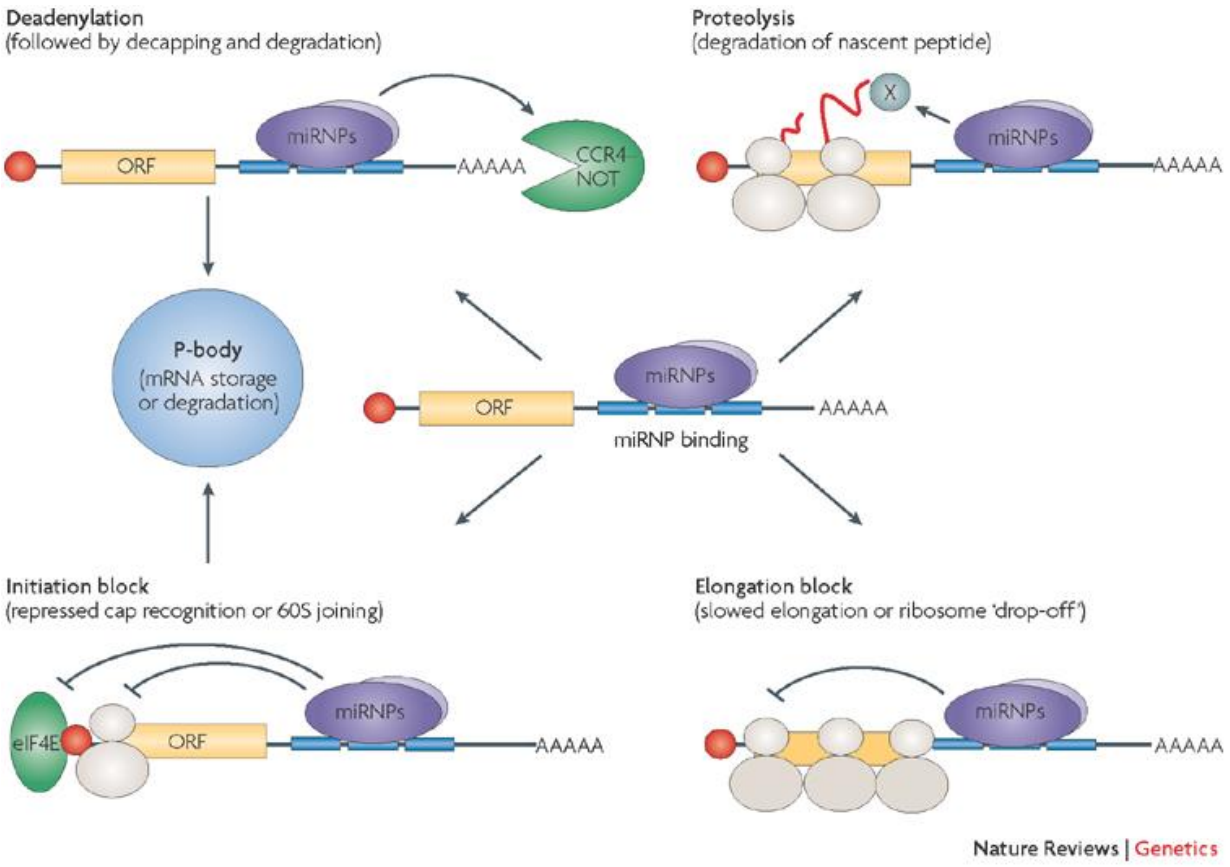
Increased tau protein production

~~Aberrant transcriptional regulation?~~

Aberrant post-transcriptional regulation?

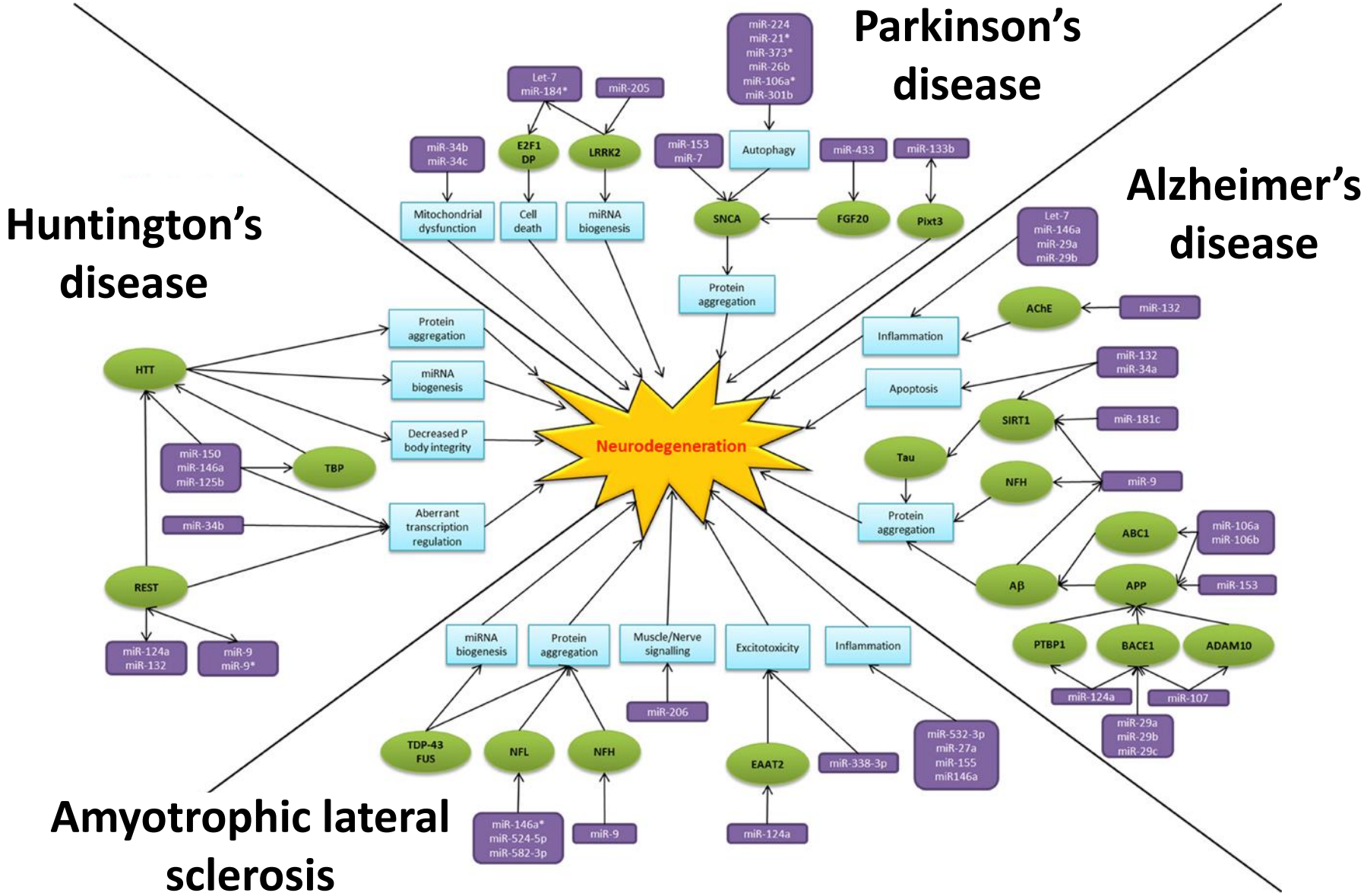
microRNAs?

Background: Post-transcriptional regulation by microRNAs

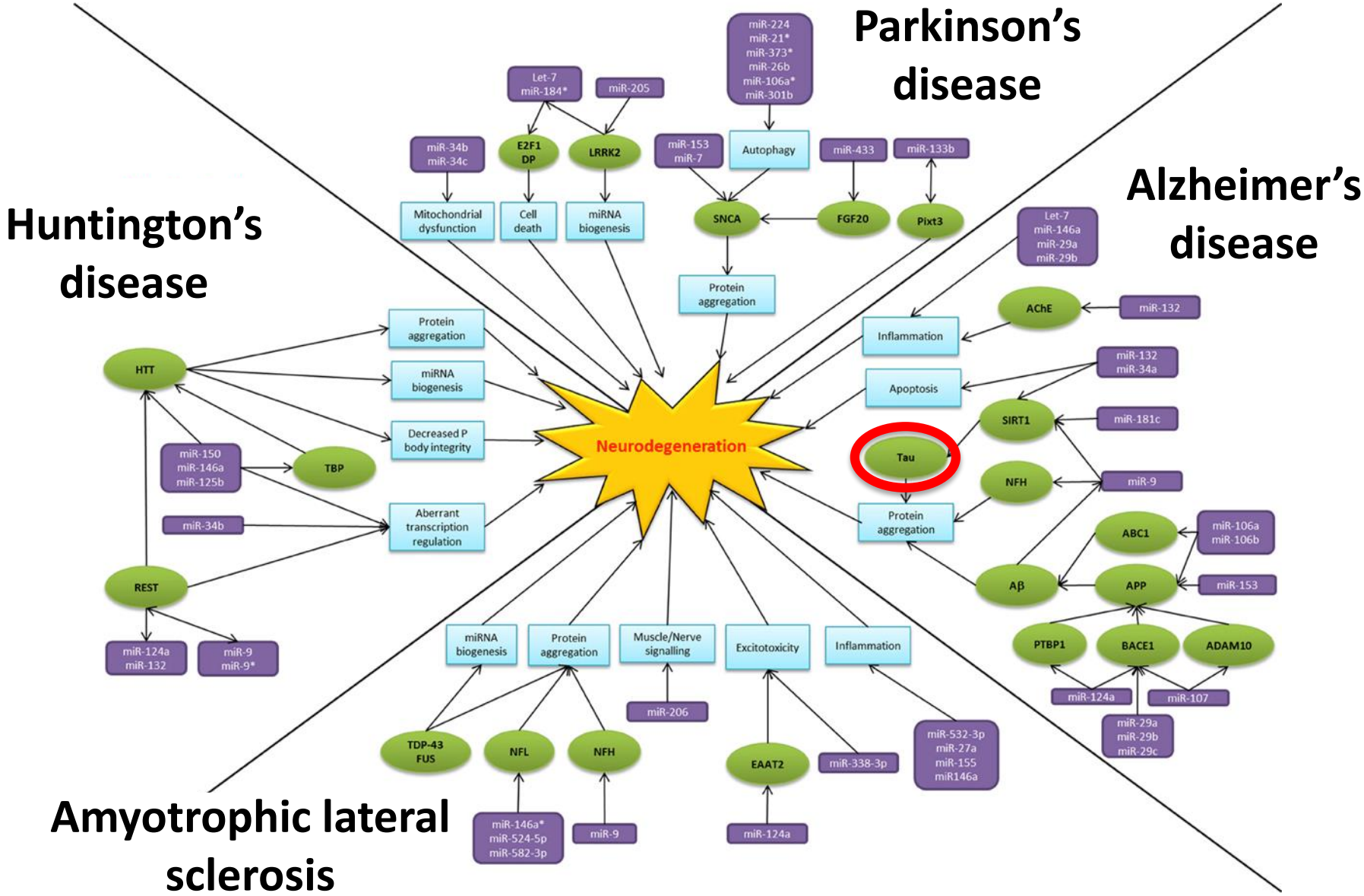


microRNAs are single-stranded RNA molecules that bind to mRNA to either degrade the mRNA or block translation

Background: microRNAs in neurodegeneration



Background: microRNAs in neurodegeneration



Role of microRNAs in tau associated neurodegeneration

→ **Are microRNAs targeting tau altered in aging and AD?**

→ **Do microRNAs modulate tau pathology?**

→ **Do microRNAs directly regulate tau synthesis?**

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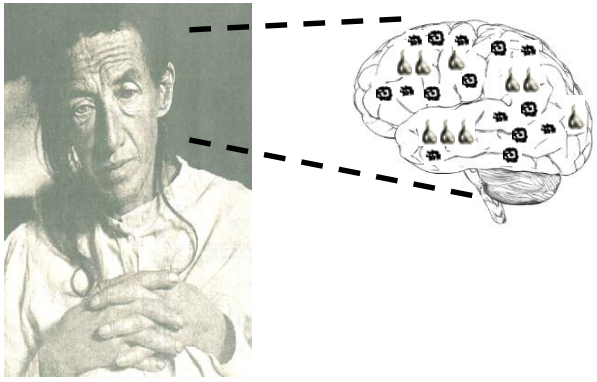
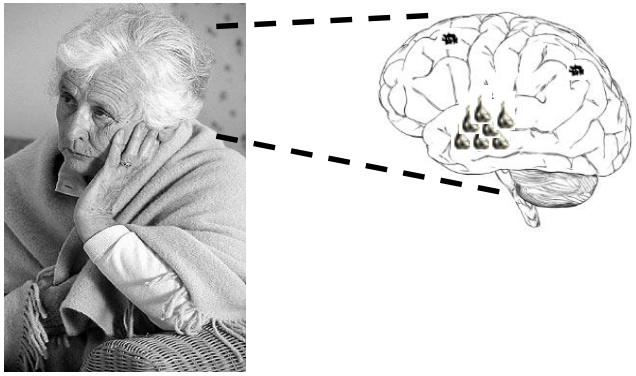
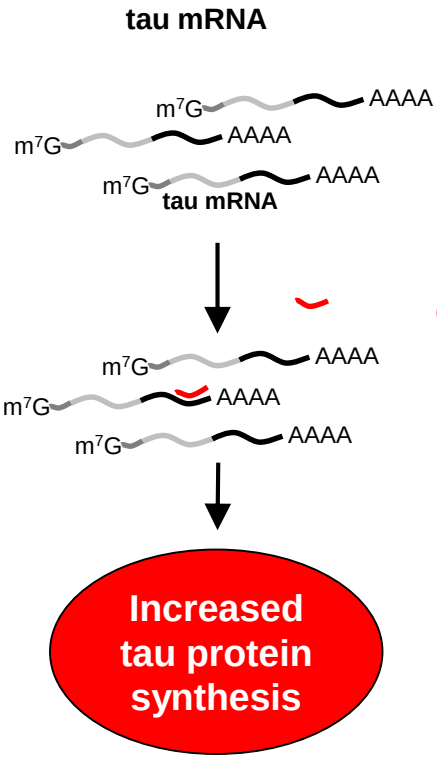
MicroRNAs and neurofibrillary degeneration



Alzheimer's disease



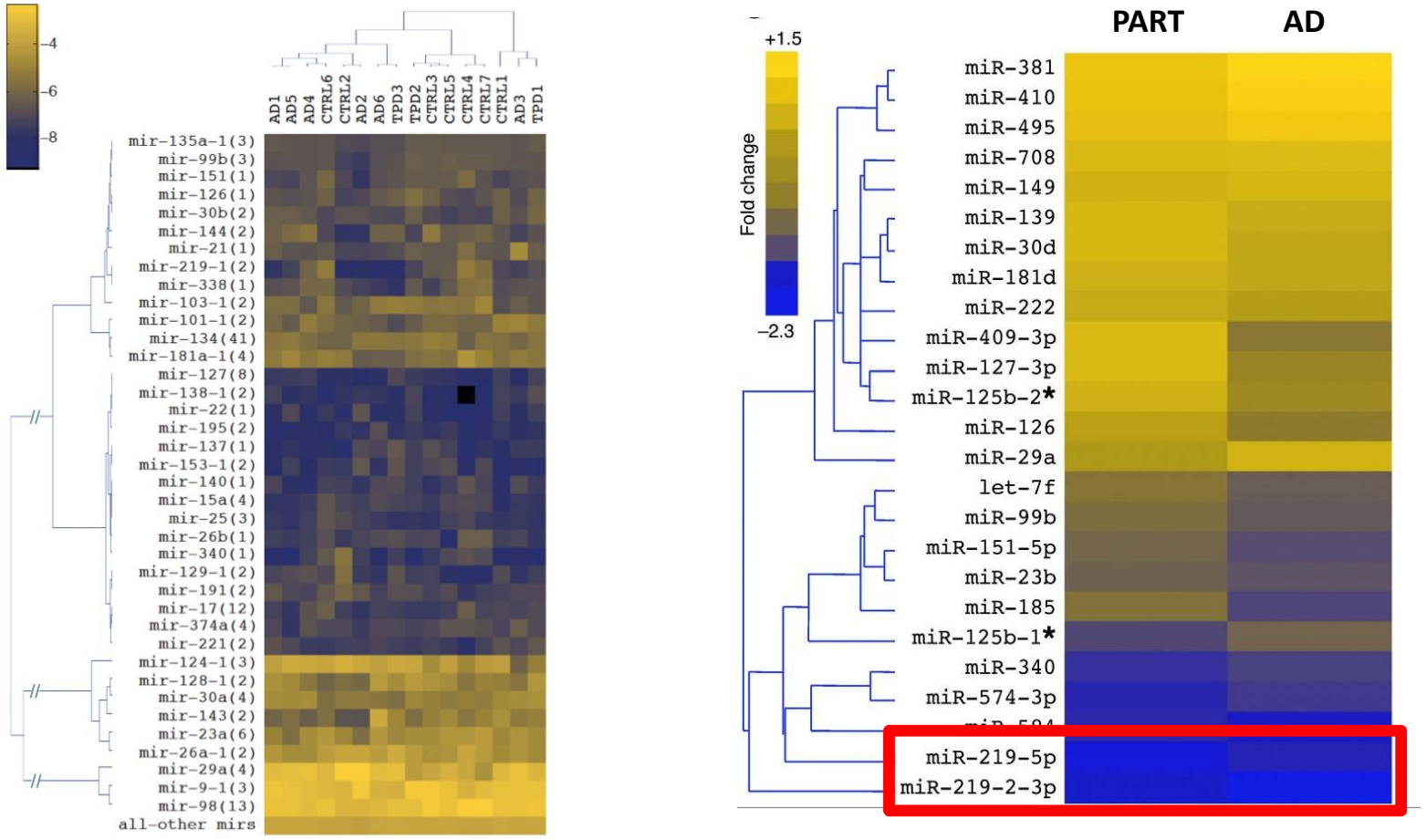
Primary-age related tauopathy
(a common pathology associated with human aging)



MicroRNA dysregulation??

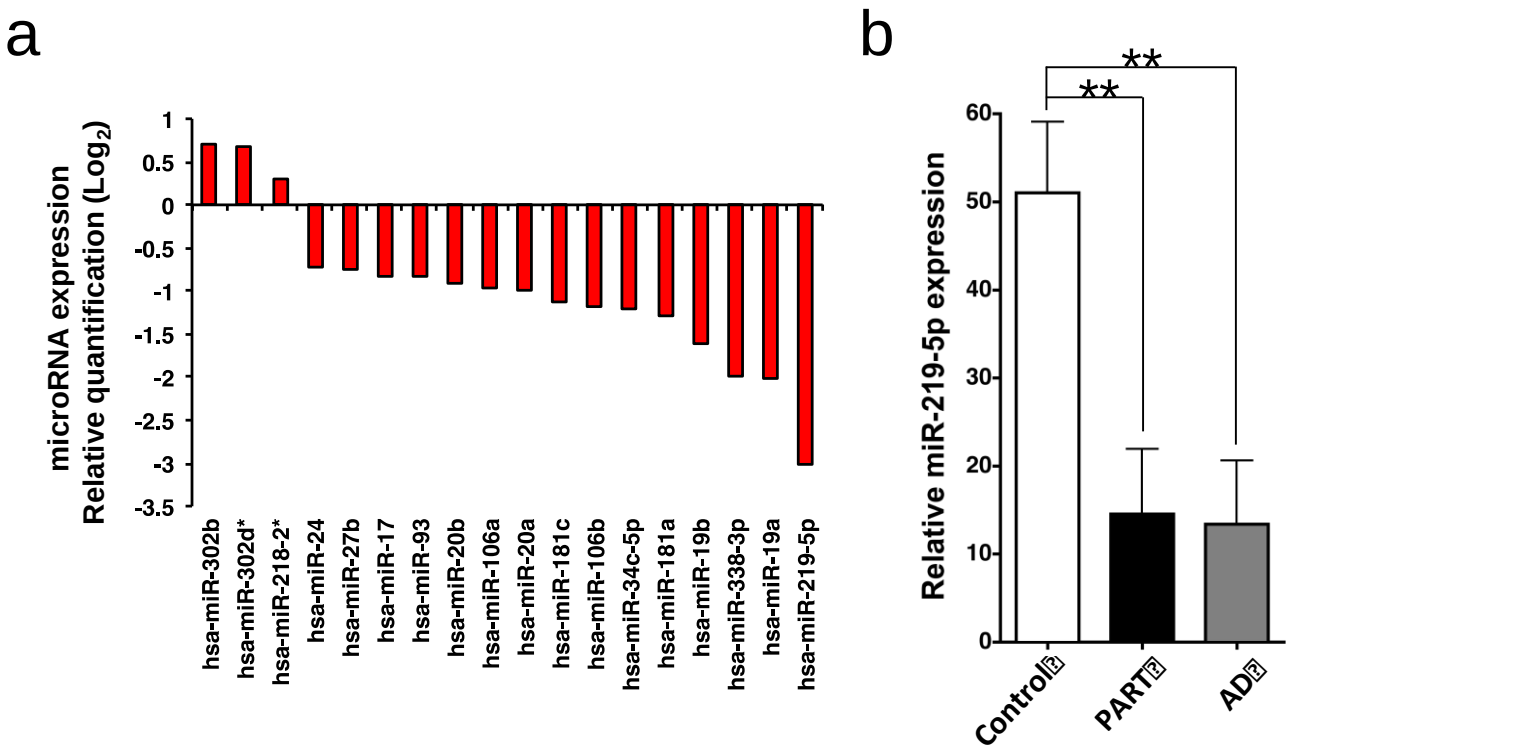
PART patients display tau inclusions/ aggregates that are regionally, morphologically, ultrastructurally and biochemically similar to those in moderate-stage Alzheimer disease

MicroRNA alterations in Aging and Alzheimer Disease



MicroRNA profiling: small RNA-seq in Alzheimer's disease (AD) and primary age-related tauopathy (PART)

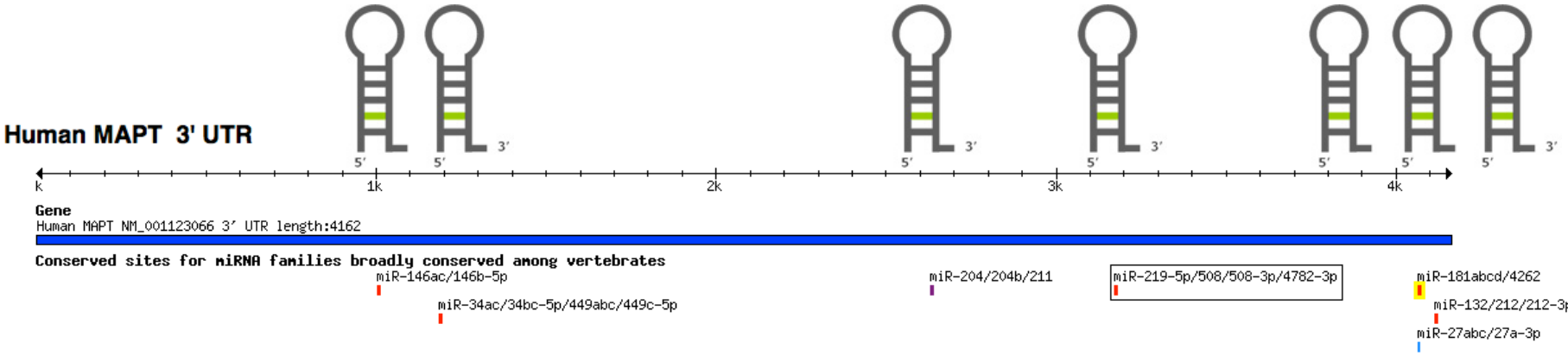
MicroRNA alterations in Aging and Alzheimer Disease



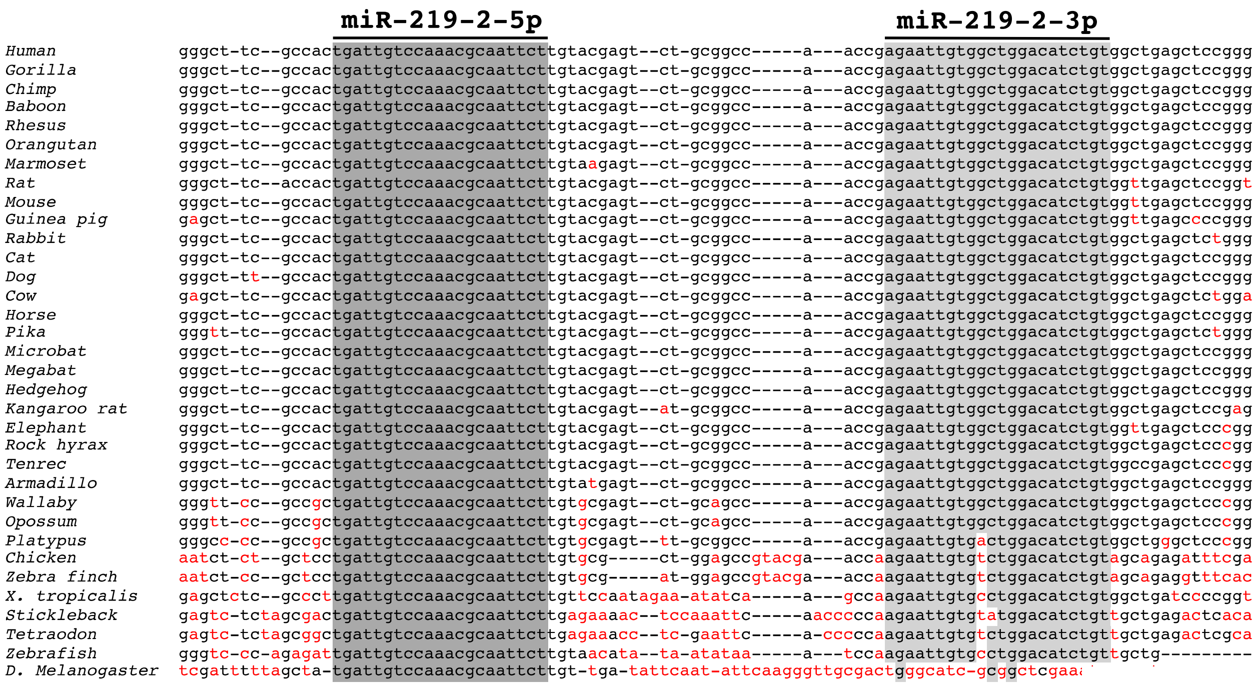
***P*< 0.01 (t-test)

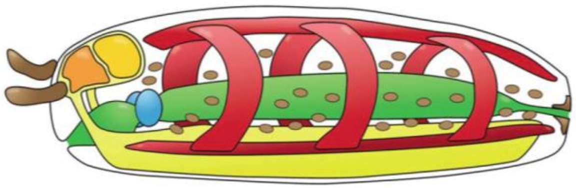
Validation of miR-219-5p expression in Alzheimer's disease (AD) and primary age-related tauopathy (PART) by High-throughput and TaqMan QPCR

Predicted human microRNAs targeting tau



MiR-219 is an ancient highly conserved miRNA in the Central Nervous System





Ancient bilaterian miRNAs

Foregut	Motile cilia	Neurosecretory brain tissue	Sensory brain tissue	General musculature	General CNS	Sensory organs	Gut	Other
miR-100 let-7^ miR-125^ miR-375^ miR-10 miR-278 miR-31	miR-29 miR-92 miR-34	miR-7 miR-137 miR-153	miR-9 miR-9*	miR-22# miR-1 miR-133	miR-71 miR-124 miR-184 miR-219	miR-8 miR-183 miR-263 miR-252^ miR-2001	miR-216 miR-283	miR-315 miR-281 miR-210^ miR-33

Foteini et al., (2010). Ancient animal microRNAs and the evolution of tissue identity. Nature 463, 1084-1088

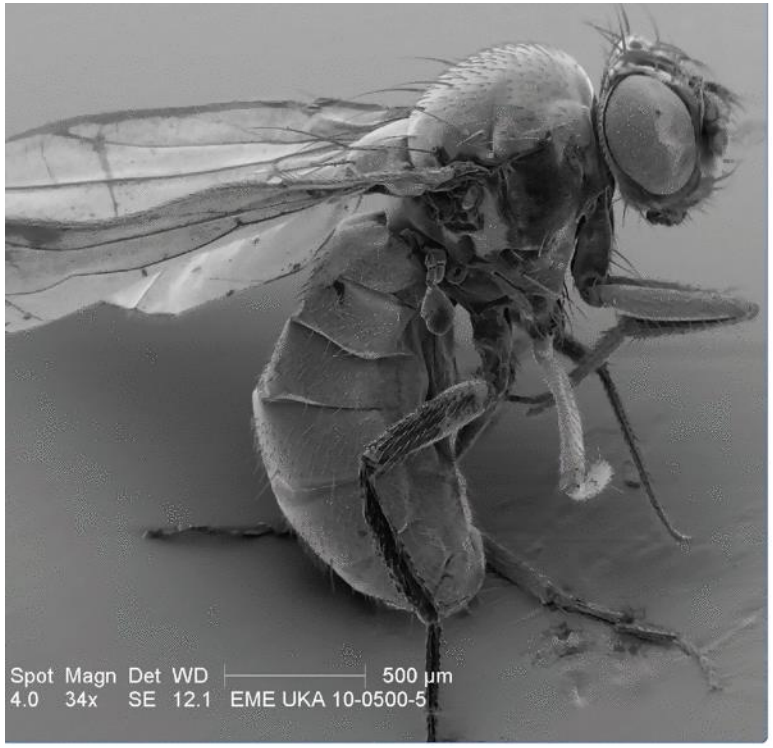
Role of microRNAs in tau associated neurodegeneration

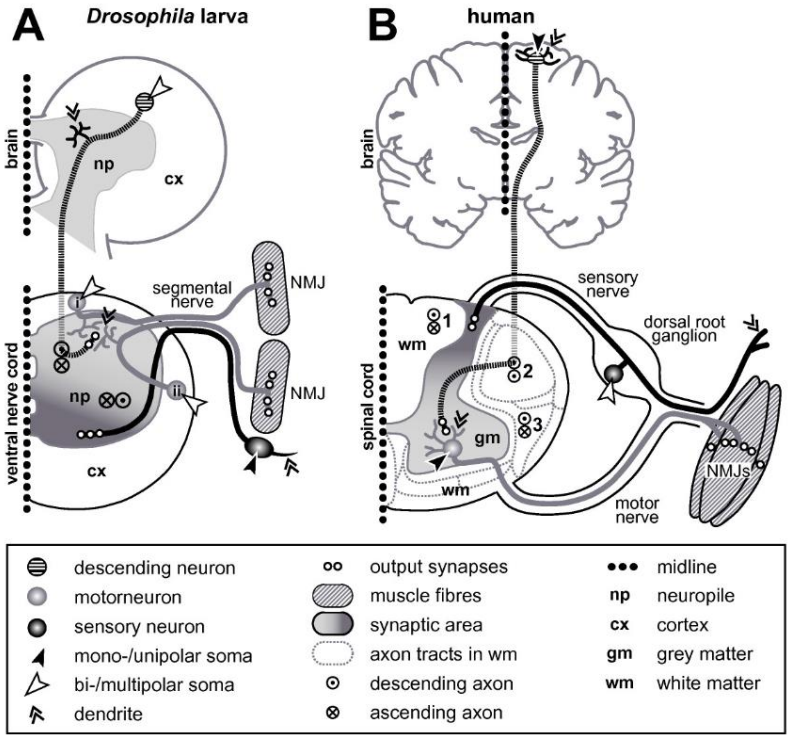
→ Are microRNAs targeting tau altered in AD?

→ **Do microRNAs modulate tau pathology?**

→ Do microRNAs directly regulate tau synthesis?

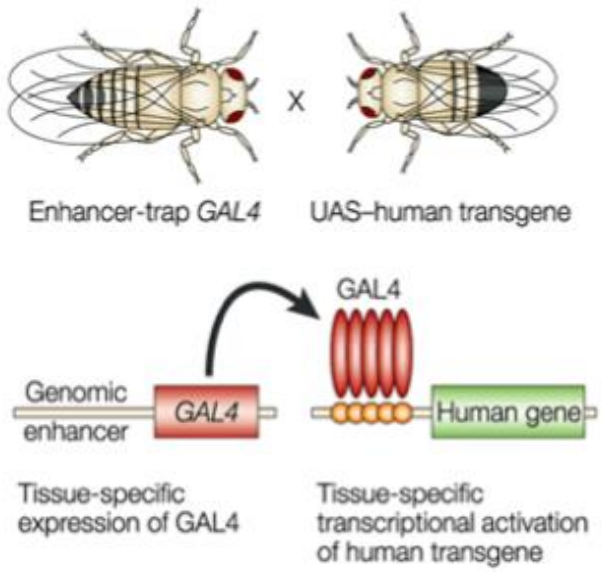
Role of miR-219 in tau induced toxicity in vivo

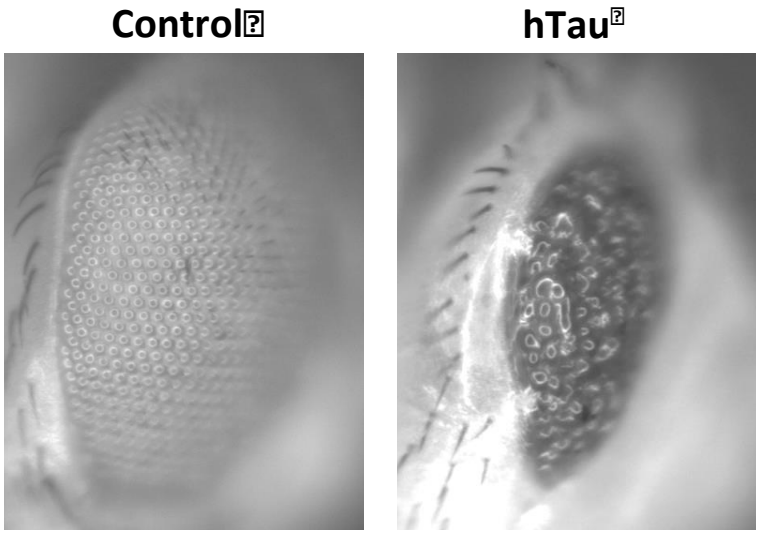




In vivo regulation of tau toxicity by miR-219

Human tau promotes neurodegeneration in vivo





GMR-Gal4 driver. Adult male flies (1 day old)

Role of miR-219 in tau induced toxicity in vivo

Development. 2012 Aug;139(15):2821-31. doi: 10.1242/dev.079939. Epub 2012 Jun 28.

A genome-wide transgenic resource for conditional expression of Drosophila microRNAs.

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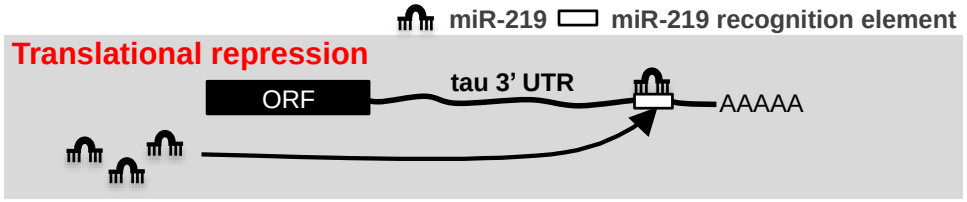

Nat Methods. 2009 Dec;6(12):897-903. doi: 10.1038/nmeth.1402. Epub 2009 Nov 15.

Transgenic microRNA inhibition with spatiotemporal specificity in intact organisms.

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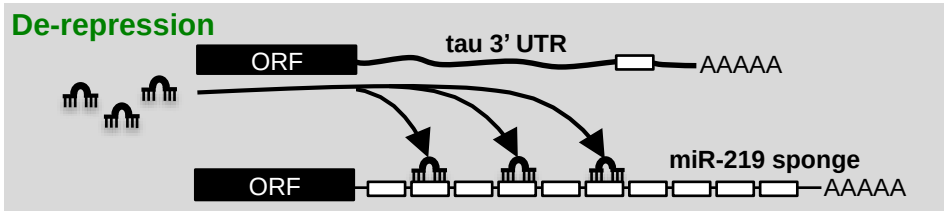

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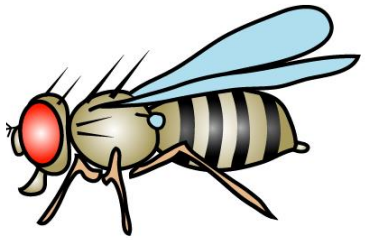
P♀



$$\frac{\text{GMR (W+)}}{\text{GMR (W+)}} \;;\; \frac{+}{+} \;;\; \frac{+}{+} \quad \mathbf{X}$$

♂

$$\frac{\text{W-}}{\text{Y/W-}} \;;\; \frac{\text{hTau (W+)}}{\text{hTau (W+)}} \;;\; \frac{\text{miR-219(W+)}}{\text{miR-219(W+)}}$$

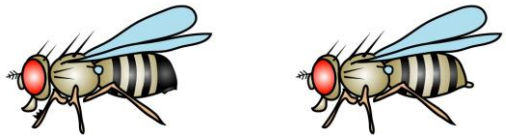
$$\frac{\text{W-}}{\text{Y/W-}} \;;\; \frac{\text{hTau (W+)}}{\text{hTau (W+)}} \;;\; \frac{\text{Scramble miR-219 (W+)}}{\text{Scramble miR-219 (W+)}}$$

$$\frac{\text{W-}}{\text{Y/W-}} \;;\; \frac{\text{hTau (W+)}}{\text{hTau (W+)}} \;;\; \frac{\text{miR-219-sp(W+)}}{\text{miR-219-sp(W+)}}$$

$$\frac{\text{W-}}{\text{Y/W-}} \;;\; \frac{\text{hTau (W+)}}{\text{hTau (W+)}} \;;\; \frac{\text{Scramble-sp(W+)}}{\text{Scramble-sp(W+)}}$$

F1♀

i.e.⚥



$$\frac{\text{GMR (W+)}}{\text{Y/W-}} \;;\; \frac{\text{htau (W+)}}{+} \;;\; \frac{\text{miR-219 (W+)}}{+}$$

Development. 2012 Aug;139(15):2821-31. doi: 10.1242/dev.079939. Epub 2012 Jun 28.

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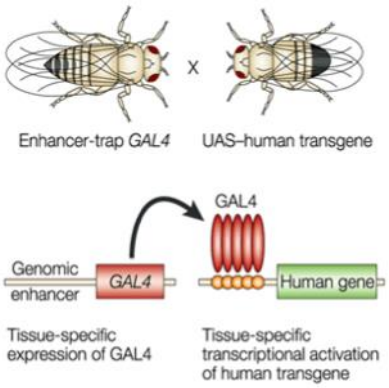
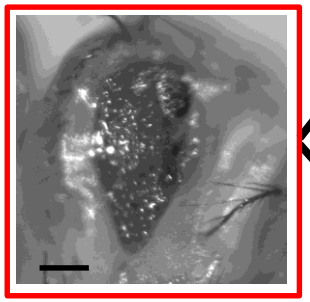
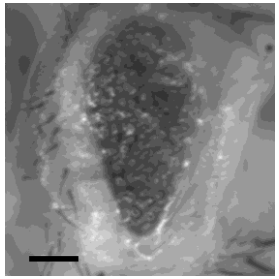


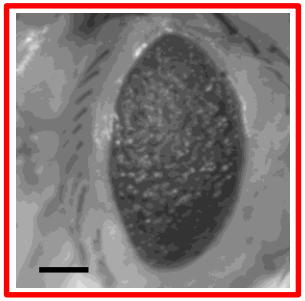


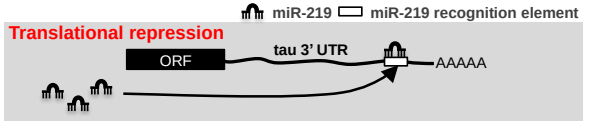
htau

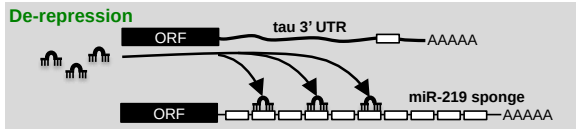
htau + scrambled miR-219

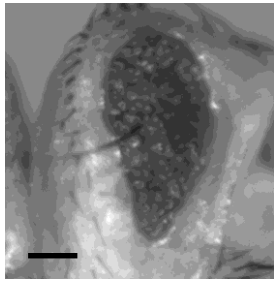


htau + miR-219

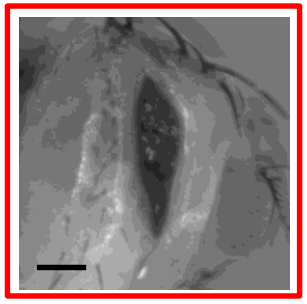




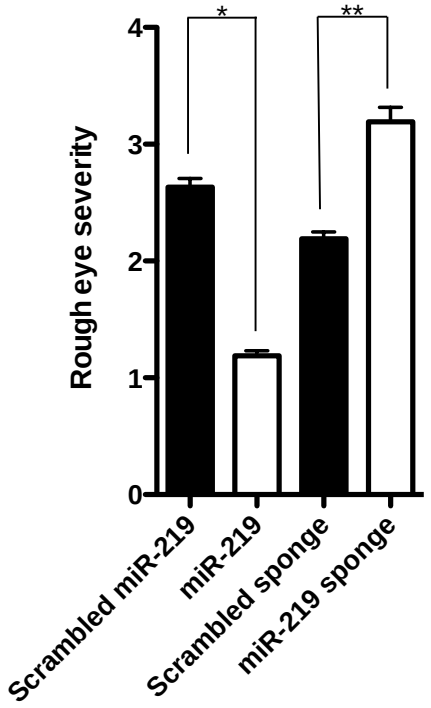




htau + miR-219 scrambled sponge



htau + miR-219 sponge



GMR-Gal4 driver
Adult male flies (1 day old)
Bar=100um

*P<0.05; **P< 0.01 (t-test); n=165

Role of microRNAs in tau associated neurodegeneration

→ Are microRNAs targeting tau altered in aging and AD?

→ Do microRNAs modulate tau pathology?

→ **Do microRNAs directly regulate tau synthesis?**

MiR-219 and its microRNA recognition element in the tau 3' UTR are conserved in Drosophila

seed

miR-219 3'-UCUUAACGCAAAC**CUGUUAG**U-5'

H. sapiens **tau 3'UTR** 5'…UCUUAAAUGAG**GACAAUC**C…3'

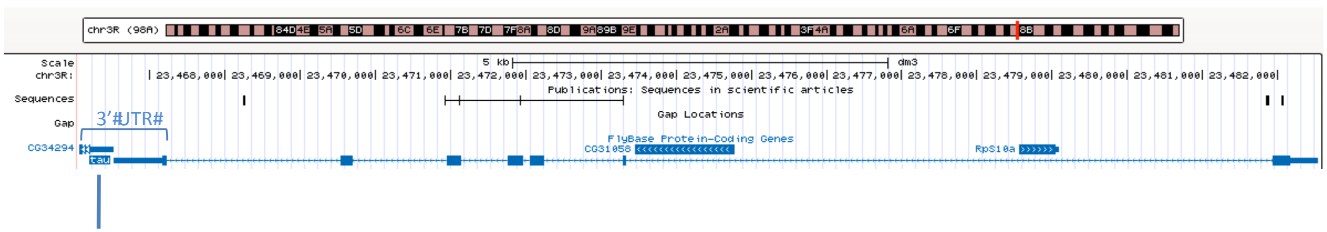
M. musculus **tau 3'UTR** 5'…ACUUCAGUGAG**GACAAUC**C…3'

R. norvegicus **tau 3'UTR** 5'…ACUUCAGUGAG**GACAAUC**C…3'

C. familiaris **tau 3'UTR** 5'…ACCUGAAUGAG**GACAAUC**C…3'

E. caballus **tau 3'UTR** 5'…CCUUAAAUGAG**GACAAUC**U…3'

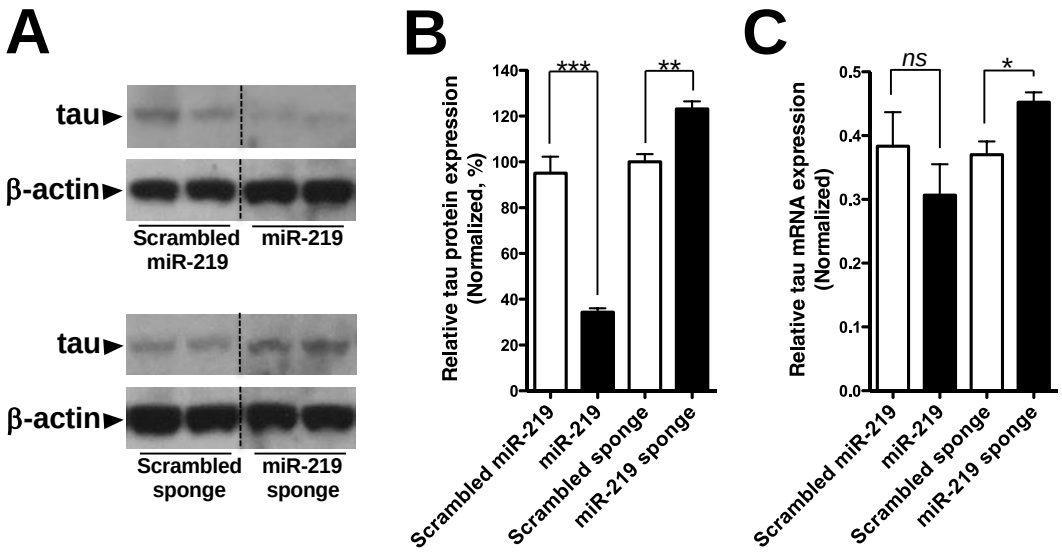
D. Melanogaster **tau 3'UTR** 5'…GGUUGAGAUA-**GACAAUC**A…3'



miR-219-5p recognition element

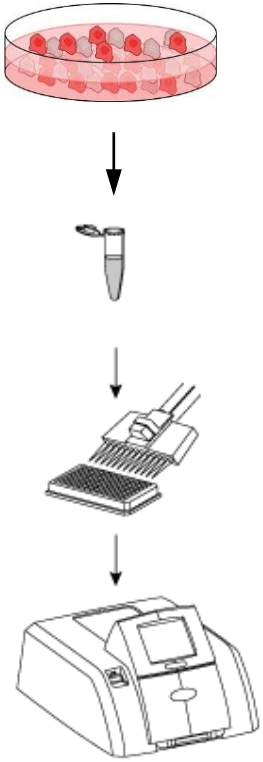
Gene_ID	miRNA_family_ID	species_ID	MSA_start	MSA_end	UTR_start	UTR_end	Group_num	Site_type
TAU	**miR$980**	7227	90	96	90	96	22	1a
TAU	**miR$33**	7227	129	136	129	135	11	8mer
TAU	miRE954	7227	164	171	164	170	16	8mer
TAU	**miR$193**	7227	182	188	182	188	4	1a
TAU	**miR$1012**	7227	304	310	304	310	2	m8
TAU	miRE33	7227	317	323	317	323	12	m8
TAU	**miR$977**	7227	356	363	356	362	21	8mer
TAU	**miR$967**	7227	357	363	357	363	20	1a
TAU	miRE33	7227	534	540	534	540	13	1a
TAU	**miR$3/309/318**	7227	561	567	561	567	9	1a
TAU	miRE219	7227	864	871	864	870	6	8mer
TAU	miRE315	7227	867	873	867	873	10	m8
TAU	miRE966	7227	889	895	889	895	19	1a
TAU	miRE961	7227	1015	1021	1015	1021	18	m8
TAU	miRE281E2E3p	7227	1056	1062	1056	1062	8	1a
TAU	miRE959	7227	1056	1062	1056	1062	17	1a
TAU	miRE8	7227	1136	1142	1136	1142	14	m8
TAU	miRE184	7227	1293	1299	1293	1299	3	1a
TAU	miRE927	7227	1421	1427	1421	1427	15	1a
TAU	miRE277	7227	1496	1502	1496	1502	7	1a
TAU	miRE1000	7227	1510	1516	1510	1516	1	1a
TAU	miRE210.1	7227	1542	1548	1542	1548	5	1a

miR-219 promotes neurodegeneration through post-transcriptional regulation of tau in Drosophila



*P<0.05; **P< 0.01; ***P< 0.001 (t-test)

MiR-219 silences tau expression through a direct interaction with the human tau 3' UTR

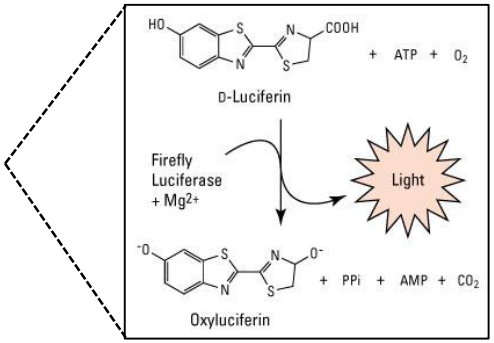


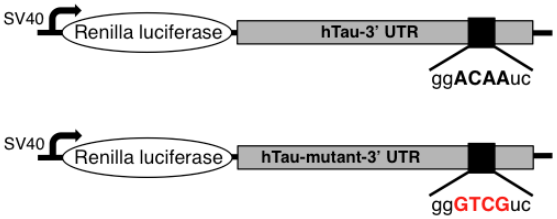
Human neuroblastoma (SH-SY5Y) cell culture

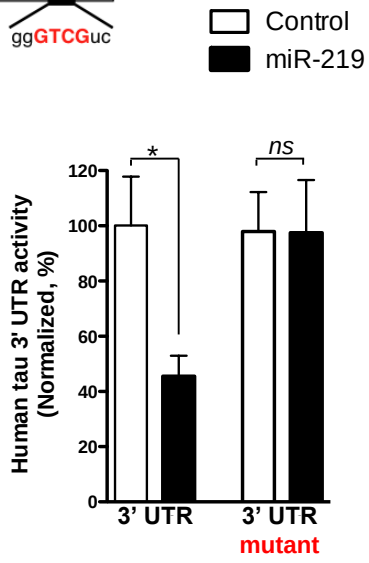
Luciferase lysate

Luciferase substrate

Luciferase activity



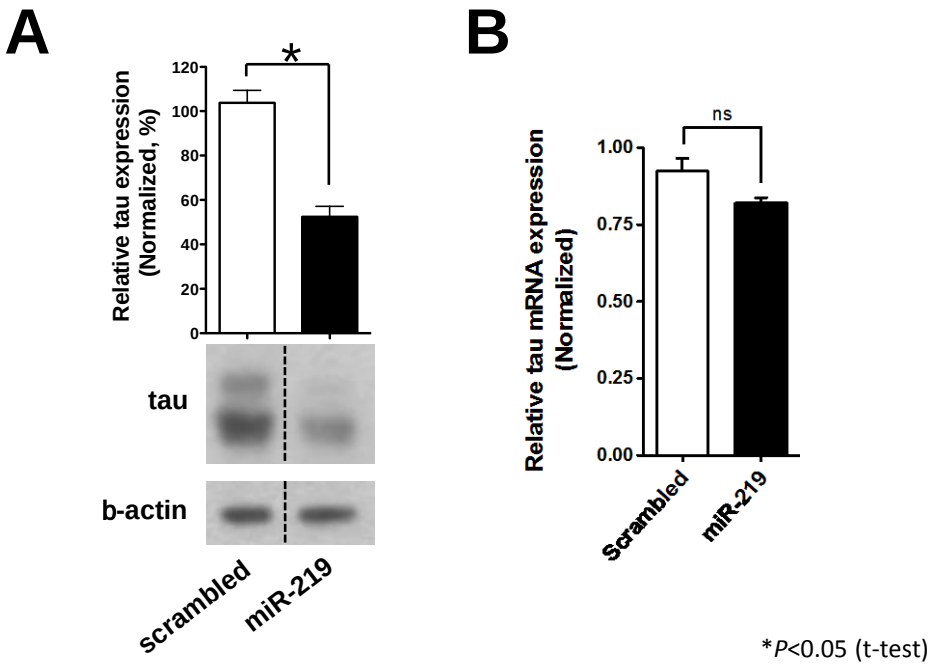




htau-3'UTR
5'…GATCTTAAATGAG**GACAATC**C…3'

htau-3'UTR-mut
5'…GATCTTAAATGAG**GGTCGTC**C…3'

Does miR-219 regulates tau in mice or rat neurons?

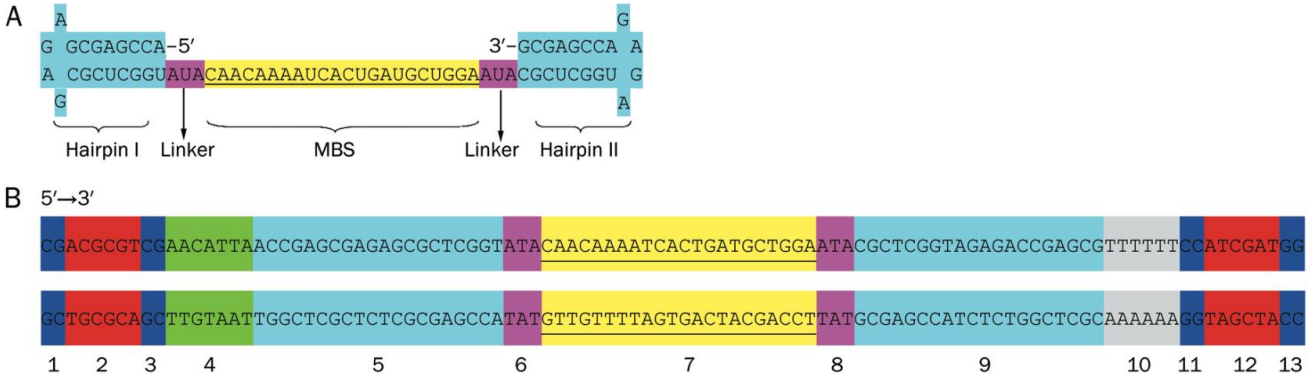


*P<0.05 (t-test)

Rat hippocampal neurons →1 week old

Generation of microRNA inhibitors: cloning strategy

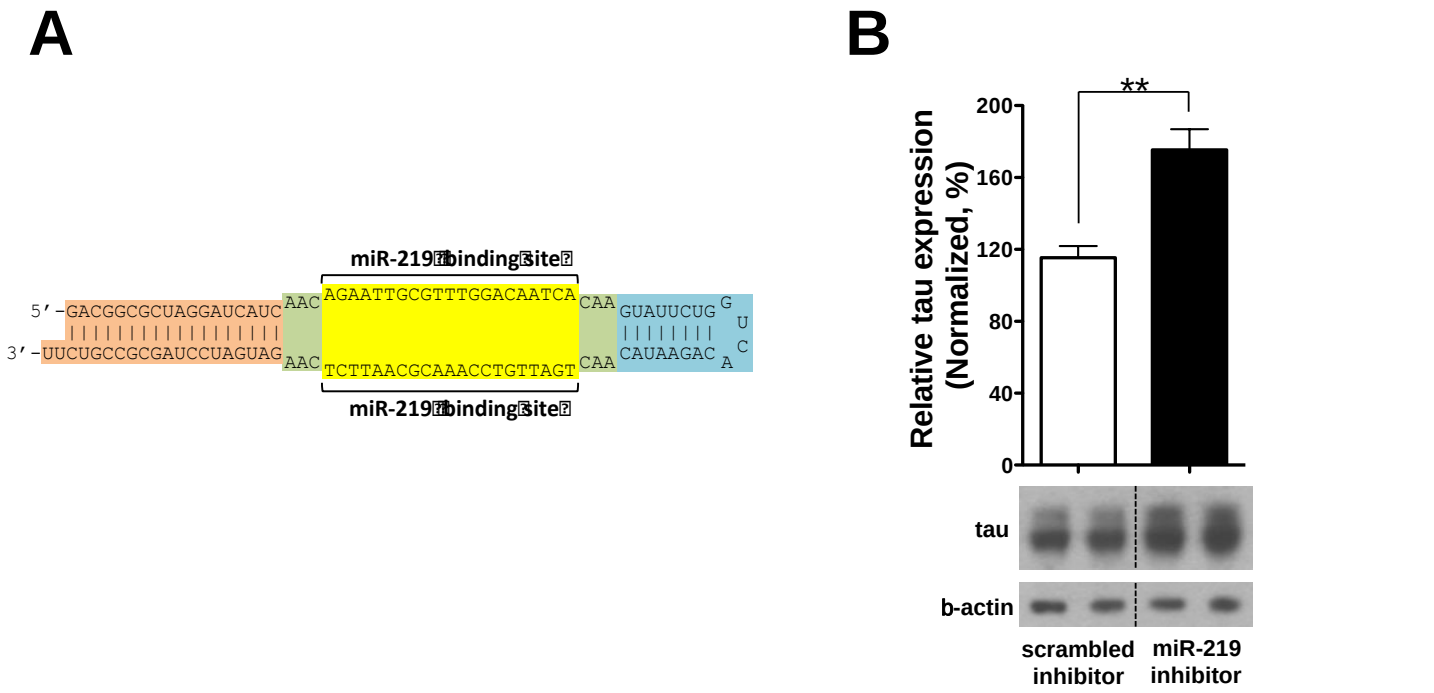
Synthetic 'Tough Decoy (TuD) RNA' molecules-miRNA inhibitors



Design of miR inhibitor sequence. (A) Representative structure of miR inhibitor. (B) Schematic representation of the generation of miR inhibitor expression cassettes driven by H1 promoter. Section 1, 3, 11, 13 are protective bases. Section 2 and 12 are restriction sites, Mlu I and Cla I respectively. Section 4 is the arbitrary nucleic acids. Section 5 and 9 are hairpin structures. Section 6 and 8 are 3-nt linkers. Section 7 is MBS. Section 10 is terminator (poly T). 81 mer synthetic oligonucleotides pairs are annealed and cloned between the Mlu I and Cla I in pLVTHM sites to generate RNA inhibitor

Haraguchi et al., 2012. Nucleic Acids Research. 40 (8):58

Does miR-219 regulates tau in mice or rat neurons?



**P< 0.01 (t-test)

Rat hippocampal neurons →1 week old

Summary

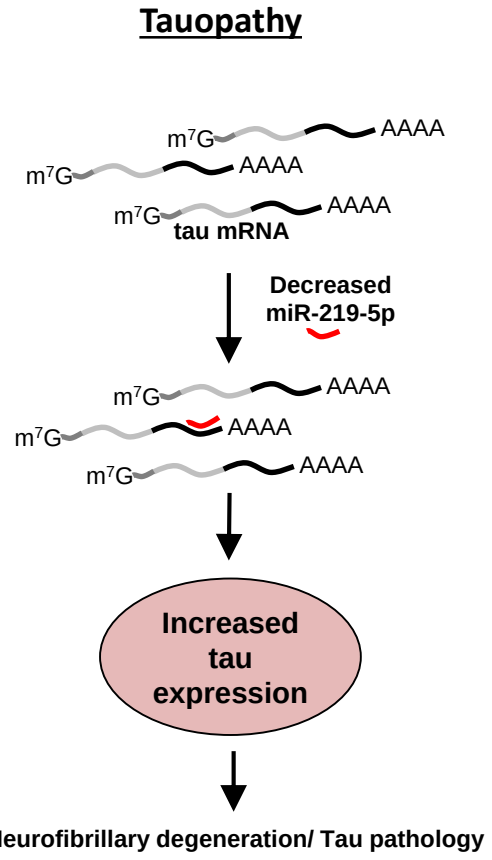
- miR-219 is an ancient CNS microRNA that is dysregulated in Alzheimer's disease and age-related tauopathy
- miR-219 modulates tau toxicity *in vivo*
- miR-219 silences tau expression through a direct interaction with a highly conserved recognition element in the tau 3' UTR

Unsolved questions

How, when, where are miR-219 levels regulated in aging and AD?

Is miR-219 targeting other proteins associated with tau physiology and pathology?

Does miR-219 regulates tau in mice *in vivo*?



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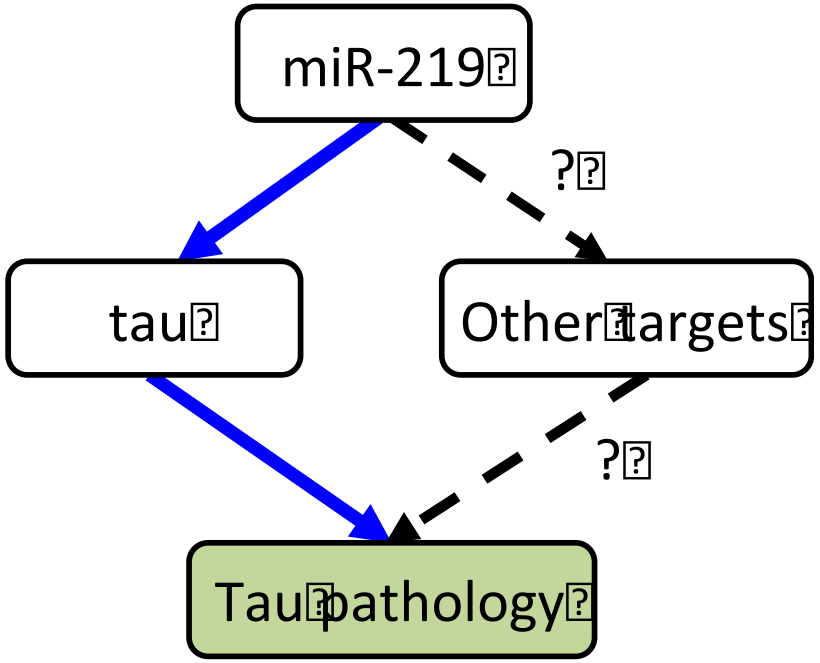
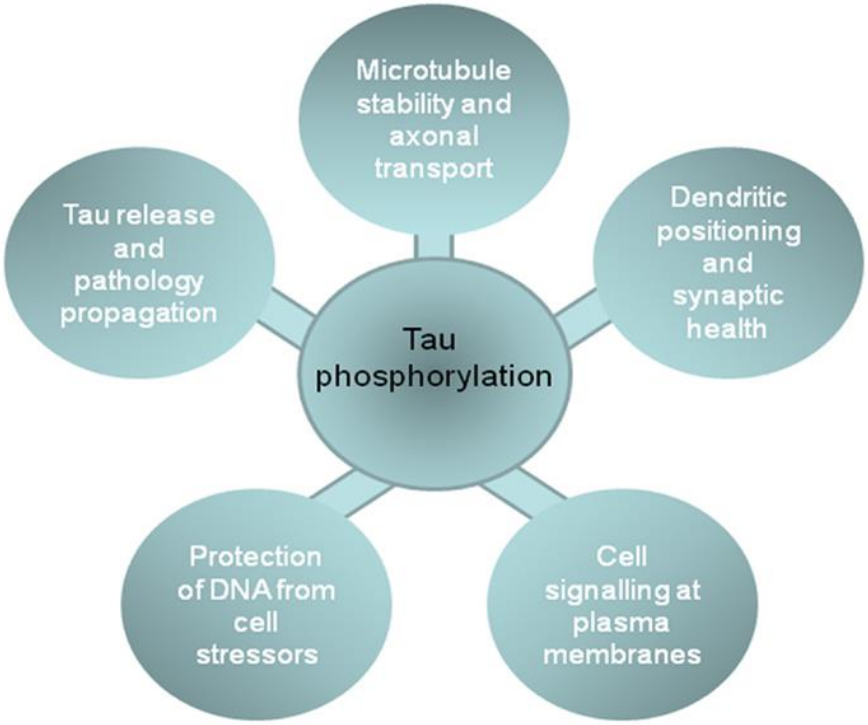


Does miR-219 regulates tau in mice or rat neurons?

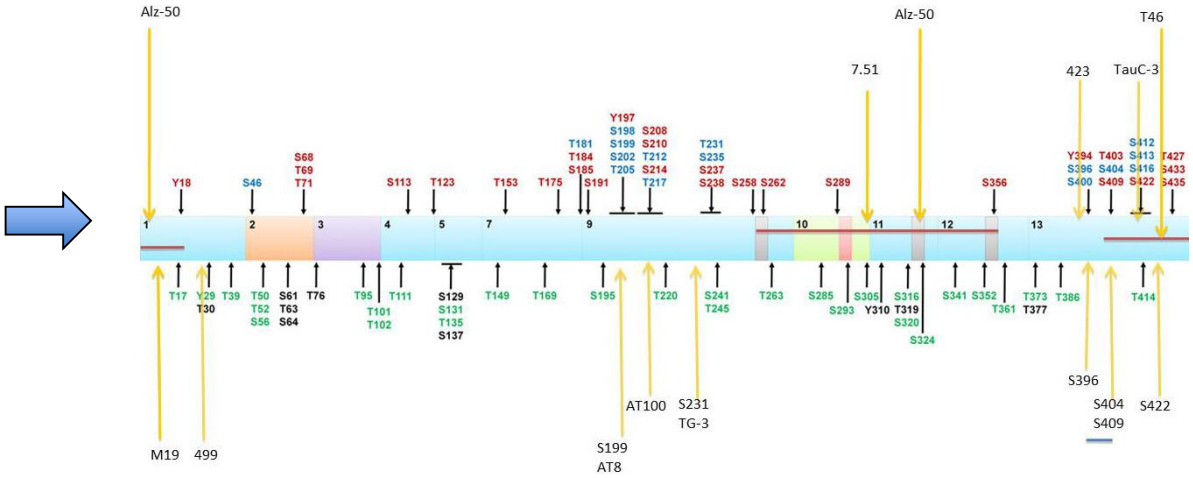
MicroRNA-mediated post-transcriptional regulation of tau pathology

Hebert SS, Papadopoulou AS, Smith P, Galas MC, Planel E, Silahtaroglu AN, Sergeant N, Buee L, De Strooper B (2010) **Genetic ablation of Dicer in adult forebrain neurons results in abnormal tau hyperphosphorylation and neurodegeneration.** Hum Mol Genet 19: 3959-3969

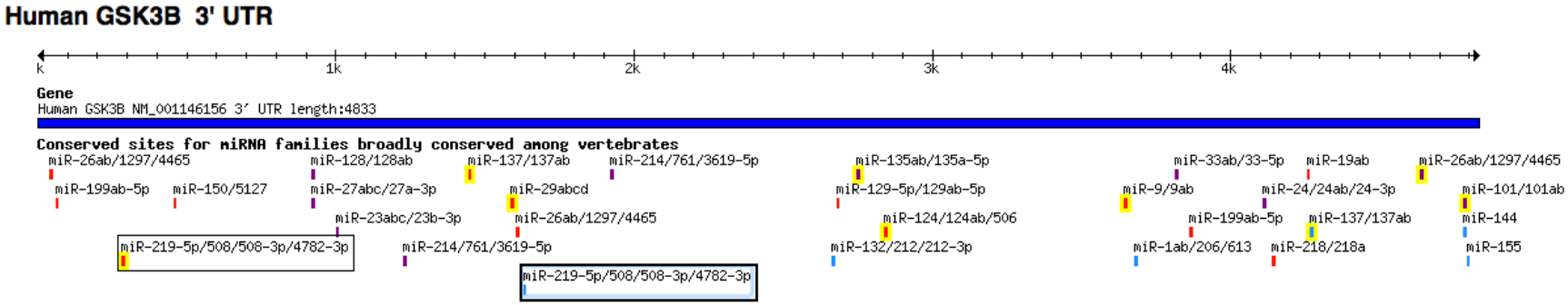


MicroRNA-mediated post-transcriptional regulation of tau pathology

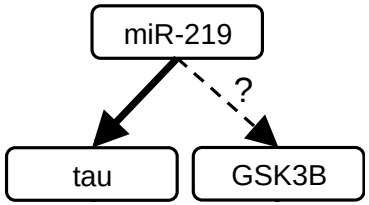
GSK-3β

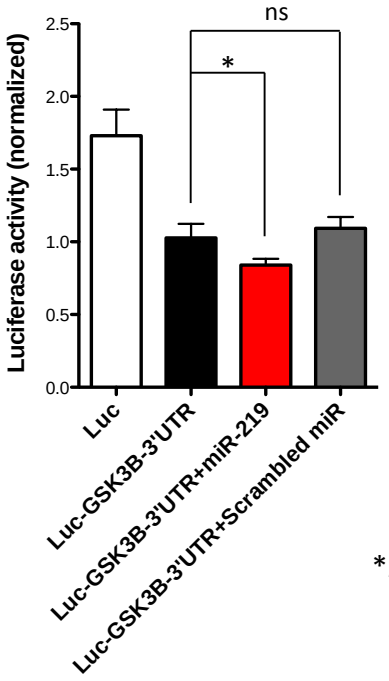


More than 30 sites on tau are phosphorylated by GSK-3β

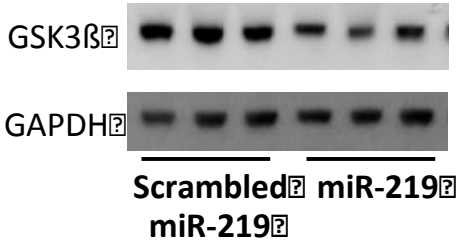


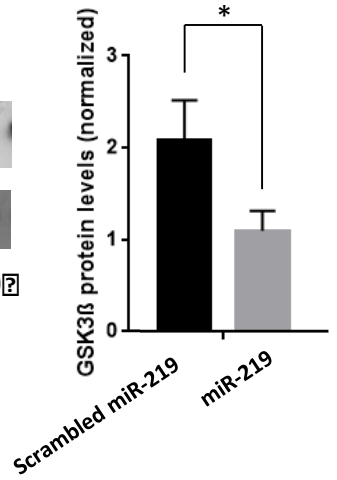
MicroRNA-mediated post-transcriptional regulation of tau phosphorylation





*P<0.05 (t-Test)

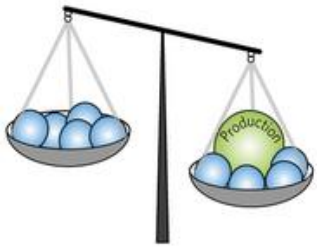




*P<0.05 (t-Test)

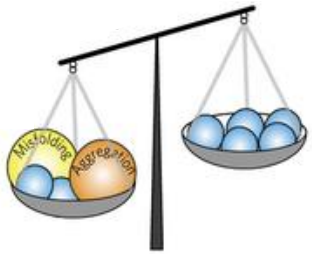
Background: Neurofibrillary degeneration

Tau protein production



→ Increased tau transcription
→ Alterations in tau splicing
→ Increased tau synthesis

Post-translational modifications



→ Tau misfolding
→ Decreased protein degradation

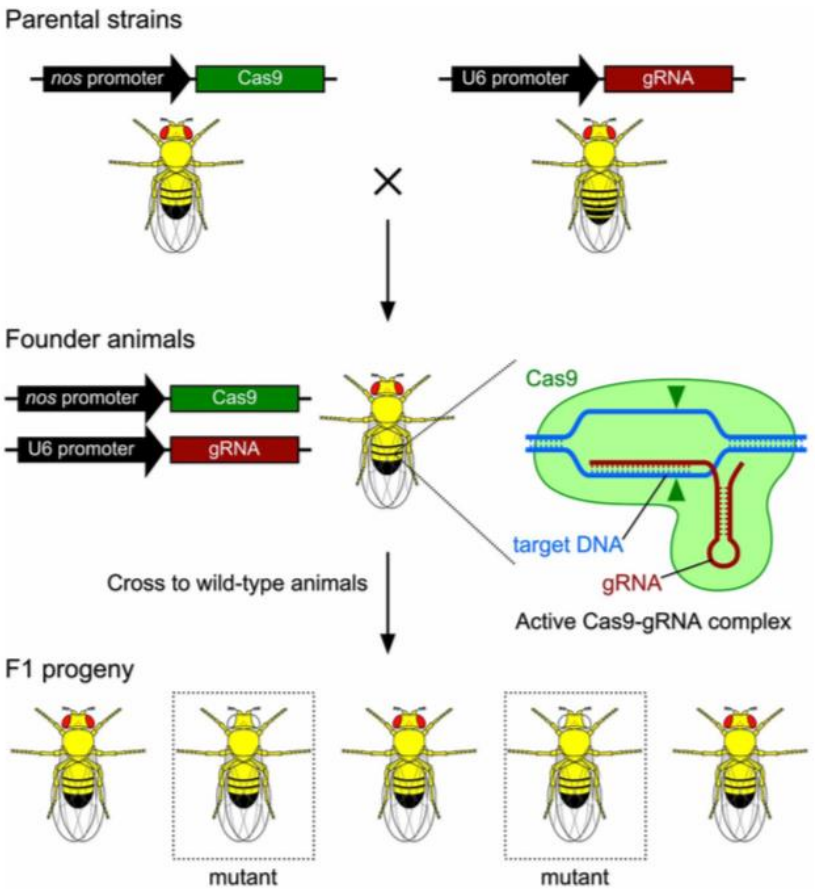
Imbalanced state of tau proteostasis

Ongoing research

→ **miR-219 mediated regulation of tau proteostasis *in vivo* in mice**

→ **Generation and characterization of miR-219 mutant Drosophila and mice lines**

miR-219 mutant *Drosophila* lines: CRISPR strategy



miR-219 gene targeting by germline-specific Cas9 expression in Drosophila

Drosophila miRNA mutants by targeted homologous recombination

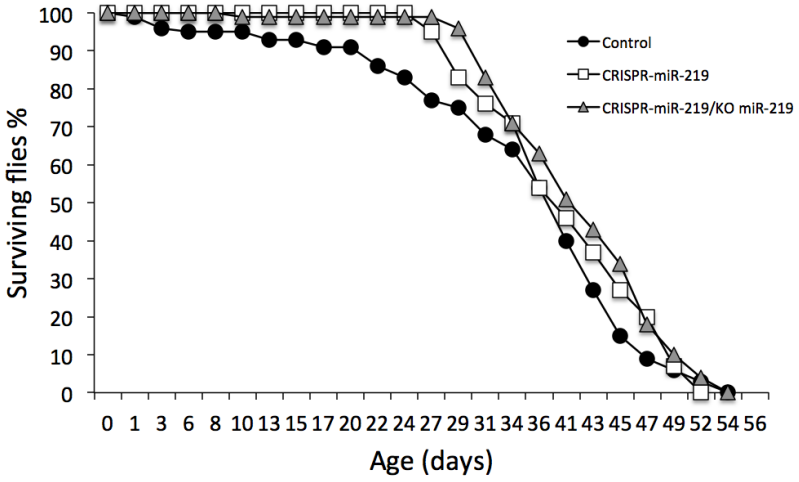
Developmental Cell
Resource

Systematic Study of *Drosophila* MicroRNA Functions Using a Collection of Targeted Knockout Mutations

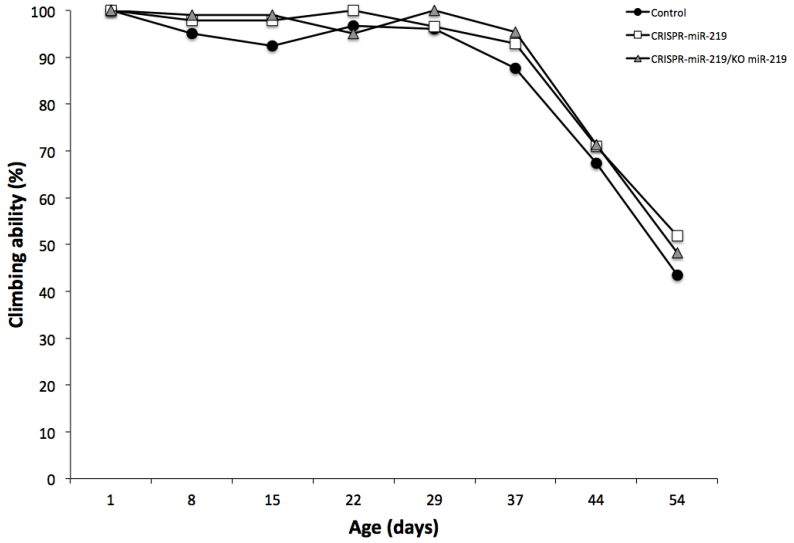
Ya-Wen Chen,[1] Shilin Song,[1] Ruifen Weng,[1] Pushpa Verma,[1] Jan-Michael Kugler,[1,4] Marita Buescher,[1,5] Sigrid Rouam,[3] and Stephen M. Cohen[1,2,4,*]

miR-219 mutant *Drosophila* lines fitness

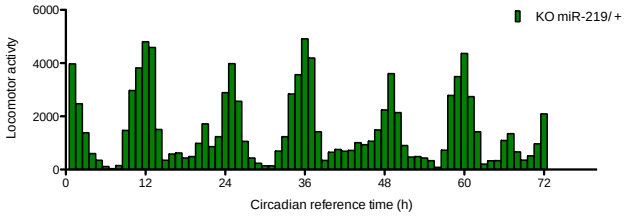
Lifespan

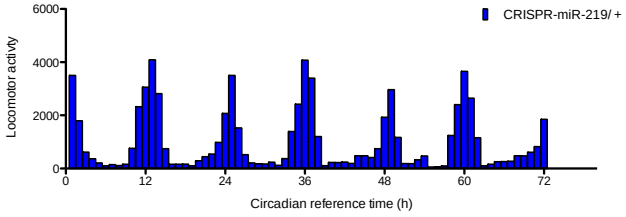


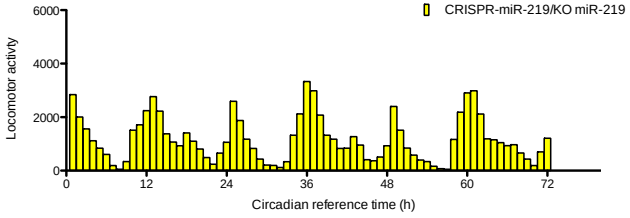
Locomotion

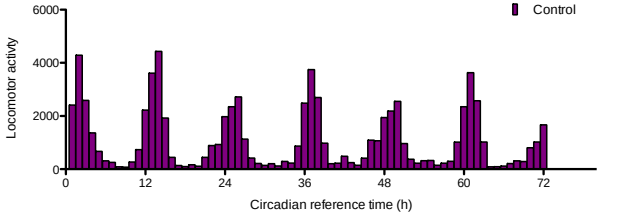


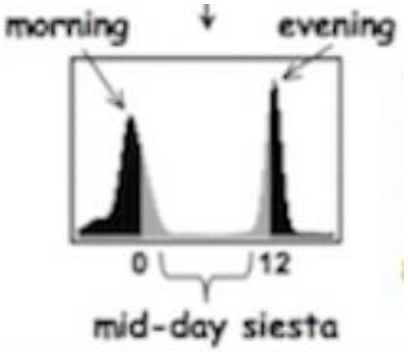
miR-219 mutant *Drosophila* lines: Circadian activity-Sleep

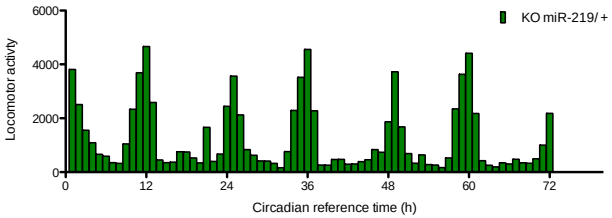


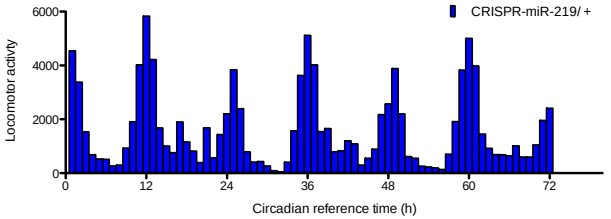


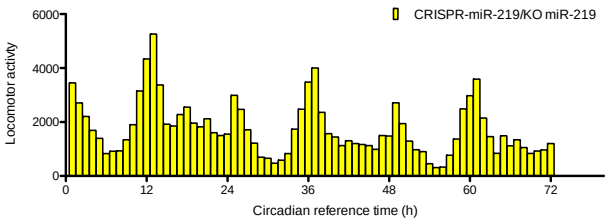


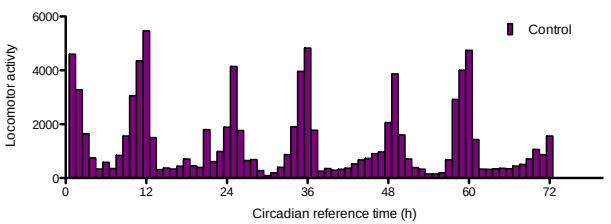










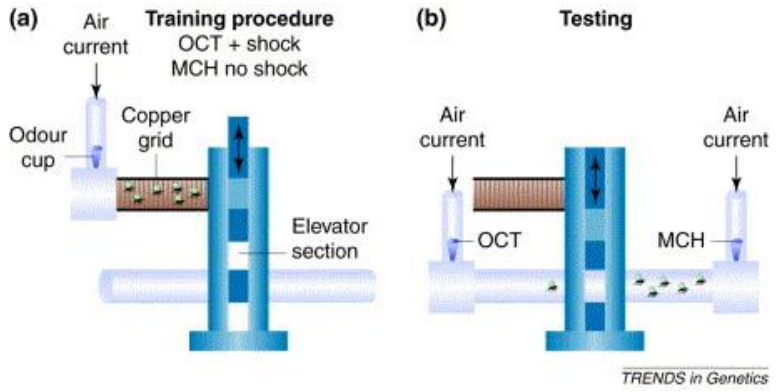


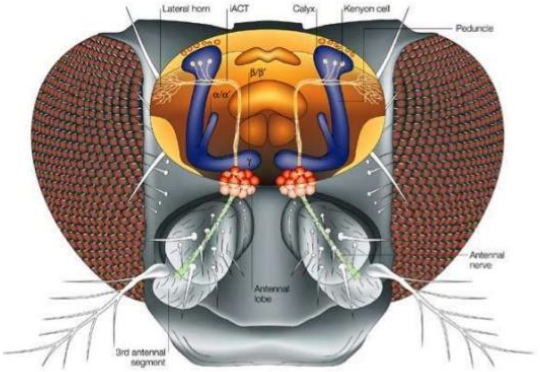
5 days old adult flies

25 days old adult flies

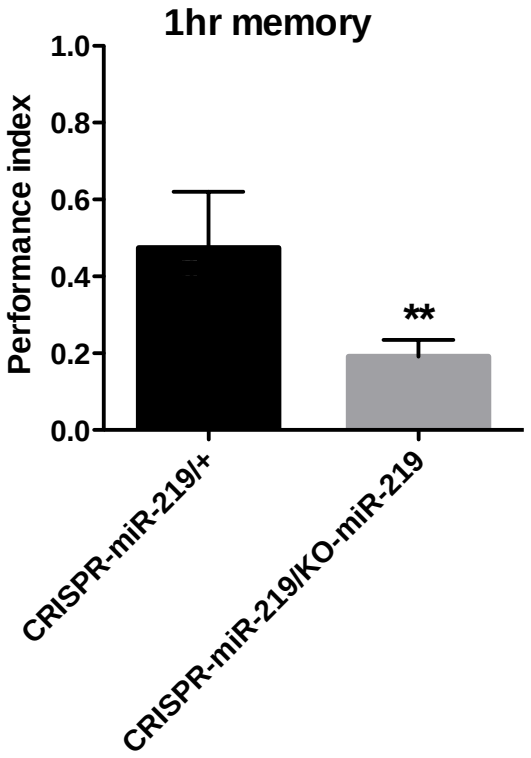
miR-219 mutant *Drosophila* lines: Associative memory

Drosophila Adult Olfactory Shock Learning





Drosophila MUSHROOM BODY



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BrightFocus Foundation

alzheimer's association