The *Tubulin Tyrosine Ligase Like 5* Gene of *Drosophila melanogaster*

Thibault Schowing

January 9, 2020

Date Performed:	HS 2019
Course:	Molecular Biology for non-biologists
Professor:	Ruth Doerig
Institution:	University of Bern

Objective

Microtubules are cytoskeletal filaments involved in movement, transport and structure of the cell. Many of these functions require post-translational modifications that regulate the activity, localization or stability of the microtubules, e.g. polyglutamylation (Schaletzky and Rape (2016)). Altering the functional property of microtubules can also alter the complex cell architecture and thus alter its functionality.

The *TTLL5* gene encode for a polyglutamylase that modifies α-tubulin. Mutated *TTLL5* is known to be involved in cone-rod degeneration and reduced male fertility in human (Bedoni et al. (2016)). The aim of this practical is to find more information about the human *TTLL5* (Q6EMB2) homolog in *D. melanogaster*. Therefore, four experiments were performed.

Experiments

Experiment 1: Fertility test Does the overexpression of *TTLL5* result in female sterility?

Experiment 2: Confocal microscopy Is the Staufen protein localization in early oocytes dependent on a functional TTLL5 protein?

Experiment 3: Western blot Does the glutamylation of α-tubulin depend on a functional TTLL5 protein?

Experiment 4: CRISPR/Cas9 Introduce a point mutation into the TTLL5 gene by using the CRISPR/Cas9 system.

Fly stocks

$$\frac{w}{w};\frac{Driver}{(Sm,Cy)};\frac{TTLL5^{PBac}}{TM,Sb}$$

$$\frac{w}{w};\frac{Driver}{(Sm,Cy)};\frac{TTLL5^{Minos}}{TM,Sb}$$

$$\frac{w}{w};\frac{Driver}{(Sm,Cy)};\frac{TTLL5^{MI-Ex}}{TM,Sb}$$

$$\frac{w}{w};\frac{venus\text{–}TTLL}{(Sm,Cy)};\frac{Df(TTLL)}{TM,Sb}$$

$$\frac{w}{w};\frac{Driver}{(Sm,Cy)};\frac{PrDr}{TM,Sb}$$

$$\frac{w}{w};\frac{venus\text{–}TTLL}{(Sm,Cy)};\frac{venus\text{–}TTLL}{TM,Sb}$$

$$\frac{w}{w};\frac{msps\text{–}mcherry}{(TM,Sb)}$$

$$\frac{w}{w};\frac{TACC\text{–}mcherry}{(TM,Sb)}$$

$$\frac{w}{w}$$

Genes and their full names:

- *TTLL5* = tyrosin tubulin ligase like 5
- Mutant alleles $TTLL^{PBac}$, $TTLL^{Minos}$, $TTLL^{Minos-Ex128}$
- *venus* = gene encoding a yellow fluorescent protein (variant of GFP)
- *mcherry* = gene encoding a red fluorescent protein
- TACC = tumor associated coiled coil protein (Used as control for fertility test)
- *msps* = mini spindles (Used as control for fertility test)

Experiment 1: Fertility test

Material and methods

Flies used for Fertility Test:

$$\frac{w}{w};\frac{venus{-}TTLL}{(Sm,Cy)};\frac{venus{-}TTLL}{Driver}$$

$$\frac{w}{w};\frac{venus{-}TTLL}{(Sm,Cy)};\frac{venus{-}TTLL}{TM,Sb}$$

$$\frac{w}{w};\frac{+}{SM,Cy};\frac{msps{-}mcherry}{Driver}$$

$$\frac{w}{w};\frac{+}{SM,Cy};\frac{TACC{-}mcherry}{Driver}$$

$$\frac{w}{w};\frac{msps{-}mcherry}{msps{-}mcherry}$$

$$\frac{w}{w};\frac{TACC{-}mcherry}{TACC{-}mcherry}$$

$$\frac{w}{w}$$

Crosses for Fertility Test:

4) $\frac{w}{w};\frac{Driver}{(SM,Cy)};\frac{PrDr}{TM,Sb}$ x $\frac{w}{};\frac{venus{-}TTLL}{(SM,Cy)};\frac{venus{-}TTLL}{TM,Sb}$

5) $\frac{w}{w};\frac{+}{+};\frac{msps{-}mcherry}{(TM,Sb)}$ x $\frac{w}{};\frac{Driver}{(SM,Cy)};\frac{+}{+}$

6) $\frac{w}{w};\frac{+}{+};\frac{TACC{-}mcherry}{(TM,Sb)}$ x $\frac{w}{};\frac{Driver}{(SM,Cy)};\frac{+}{+}$

Procedure

Material

- Apple juice plates
- Yeast

Preparation

For the apple juice plates, disolve 1 L boiling tap water with 30 g agar. Mix with 35 g white table sugar and 2 g Nipagin (Methyl-4-hydroxy-benzoate) disolved in 350 mL apple juice. Pour about 100 small or 30 medium sized plates. Store at 4 °C. Prior use, add some yeast paste.

0.0.1 Flies

- Collect females once a day
- Cross three 2-4 days old females with three *white* males and place flies into a fresh vial containing few grainsof dried yeast
- Remove adult fles after 2-3 days and wait for larvae to crawl up the glass wall
- Use the removed females for egg layings.

Figure 1:

Results

		Hatching temp of female	Layed eggs	Unhatched eggs	Hatching rate
4)	$w; \frac{venus-TTLL}{venus-TTLL}; \frac{venus-TTLL}{TM,Sb}$	25°C	100	7	93
	$w; \frac{venus-TTLL}{Driver}; \frac{venus-TTLL}{TM,Sb}$	25°C	97	9	90.72
5)	$w; \frac{msps-mcherry}{msps-mcherry}$	25°C	94	49	48
	$w; \frac{msps-mcherry}{TM,Sb}$	25°C	100	48	52
	$w; \frac{Driver}{+} \frac{msps-mcherry}{+}$	25°C	100	67	33
	$w; \frac{Driver}{+} \frac{msps-mcherry}{+}$	25°C	9	4	(56)
6)	$w; \frac{TACC-mcherry}{TACC-mcherry}$	25°C	91	29	68
	$w; \frac{TACC-mcherry}{TM,Sb}$	25°C	88	66	25
	$w; \frac{Driver}{+}; \frac{TACC-mcherry}{+}$	25°C	111	79	29
2)	$w; \frac{venus-TTLL}{SM,Cy}; \frac{Minos}{Df(TTLL)}$	29°C	30	9	70
	$w; \frac{venus-TTLL}{Driver}; \frac{Minos}{Df(TTLL)}$	29°C	73	6	92
4)	$w; \frac{venus-TTLL}{venus-TTLL}; \frac{venus-TTLL}{TM,Sb}$	29°C	86	17	80
	$w; \frac{venus-TTLL}{Driver}; \frac{venus-TTLL}{TM,Sb}$	29°C	85	14	84

Experiment 2: Confocal microscopy

Experiment 3: Western blot

Experiment 4: CRISPR/Cas9

Conclusion and discussion

References

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