CRISPR/Cas9-based genome editing of the FoxP locus in Drosophila melanogaster

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SCAN ME

1. Introduction

FoxP

The family of Fox genes groups a large number of transcription factors that share a conserved DNA-binding domain, the Forkhead domain. In vertebrates the FoxP subfamily comprises four genes differentially expressed in different tissues. In *Drosophila melanogaster* on the contrary, only one gene of the FoxP subfamily has been identified (*dFoxP*).

The Foxp2 gene sequence is highly conserved in vertebrates and has been shown to be important in vocal learning and the modulation of the underlying neural circuits. One mutation in the FOXP2 human gene affects language acquisition in afflicted patients, without other obvious impairments.

Learning

- operant world-learning, in which the subject is learns from relations between the environment
- operant self-learning, in which the subject learns from the outcome of its own behavior

The FoxP2 gene has been found to be involved in operant-self learning in various mammalian species, and the Drosophila homologous seems to have a conserved function.
However, due to contradicting evidence, the expression pattern, function and mechanisms of action remain still to be elucidated).

2. Methods

Homology recombination by CRISPR/Cas9

homology insert 1   homology insert 2

Exon
UTR
attP
Marker/Gal4
Multiple cloning site
LoxP Site

HDR

Cre

Buridan´s paradigm

Camera
Fluorescent bulbs
stripe
Platform: Ø = 117mm
arena: Ø= 293mm
Computer running the BuriTrack software

Flight simulator

Yaw torque signal
IR laser diod (heat punishment)
Projector
Flight simulator/ Torque meter
Flight arena
Light guides

3. Results

WT gene locus
1 2 3 4 5 6 7/8

Isoform unspecific driver line
1 2 3 LexA 3 4 5 6 7/8

IsoB-specific driver line
1 2 3 4 5 6 Gal-4 8

3rd instar larvae   Adult
CD8-GFPnc82   CD8-GFPnc82   CD8-GFPnc82

pSMAD1/5CD8-GFP   GABACD8-GFP   THCD8-GFP   CB
pSMAD1/5CD8-GFP   GABACD8-GFP   THCD8-GFP   VNC

- FoxP-iB is expressed at every time point during development
- FoxP-iB is expressed only in nerons and not in glia
- FoxP-iB is expressed in various types of neuronal cells, like: motorneurons, GABA- ergic, cholinergic and dopaminergic neurons

Fox P-Gal4
Fox PB-Gal4

65%
All FoxP isoforms
FoxP-iB

Relative gene expression
Ctrl
Heterozygous
Homozygous
FoxP-iA   FoxP-iIB   FoxP-iB

Buridan' s paradigm

Median speed — WTB, FoxP-iB-G4 +, FoxP-iB-G4 FoxP-iB-G4
Distance travelled — WTB, FoxP-iB-G4 +, FoxP-iB-G4 FoxP-iB-G4
Activity time — WTB, FoxP-iB-G4 +, FoxP-iB-G4 FoxP-iB-G4
Stripe deviation — WTB, FoxP-iB-G4 +, FoxP-iB-G4 FoxP-iB-G4

WTB   FoxP-iB-G4 + FoxP-iB-G4   FoxP-iB-G4 FoxP-iB-G4

Flight simulator
WTB, FoxP-iB-G4 +, FoxP3955

* p<0.05; ** p<0.01; *** p<0.001 - all graphs are represented with standard error of the mean.

The homozygous phenotype has a low flight performance, which was insufficient for the flight simulator test.

FoxP- KO
1 2 3 4 5 6 7/8

WTB   FoxP-/-

The KO construct is working well.

Buridan' s paradigm
Median speed — WTB, FoxP-LA +, FoxP-LA FoxP-LA
Distance travelled — WTB, FoxP-LA +, FoxP-LA FoxP-LA
Activity time — WTB, FoxP-LA +, FoxP-LA FoxP-LA
Stripe deviation — WTB, FoxP-LA +, FoxP-LA FoxP-LA

* p<0.05; ** p<0.01; *** p<0.001 - all graphs are represented with standard error of the mean.

FoxP- conditional KO   UAS tRNA gRNA tRNA gRNA tRNA gRNA tRNA gRNA

Buridan' s paradigm

ELAV Gal4   KO IN ALL NEURONS
Median speed — Ctrl1, Ctrl2, Exp_line — ELAV-Gal4; UAS-tgFoxP
Distance travelled — Ctrl1, Ctrl2, Exp_line — ELAV-Gal4+UAS-tgFoxP
Activity time — Ctrl1, Ctrl2, Exp_line — ELAV-Gal4+UAS-tgFoxP

ELAV Gal4   Tub Gal80   TIME SPECIFIC KO IN ALL NEURONS
Embryo 1st instar larva 2nd instar larva 3rd instar larva Pupa Adult
Median speed — Ctrl1, Ctrl2, Exp_line — ELAV-Gal4; Tub-Gal80 / ELAV-Gal4+UAS-tgFoxP Tub-Gal80
Distance travelled — Ctrl1, Ctrl2, Exp_line
Number of walks — Ctrl1, Ctrl2, Exp_line
Activity time — Ctrl1, Ctrl2, Exp_line

D42 Gal4   KO IN MOTORNEURONS
Median speed — Ctrl1, Ctrl2, Exp_line — D42-Ga l4 / UAS-tgFoxP D42-Ga l4+UAS-tgFoxP
Distance travelled — Ctrl1, Ctrl2, Exp_line
Number of walks — Ctrl1, Ctrl2, Exp_line

Embryo 1st instar larva 2nd instar larva 3rd instar larva Pupa Adult
Median speed — Ctrl1, Ctrl2, Exp_line
Distance travelled — Ctrl1, Ctrl2, Exp_line
Number of walks — Ctrl1, Ctrl2, Exp_line
Activity time — Ctrl1, Ctrl2, Exp_line

* p<0.05; ** p<0.01; *** p<0.001 - all graphs are represented with standard error of the mean.

The time specific conditional KO was done as follows: flies were grown at 18 °C, and once hatched the virgins were put at 30 °C for 48 hrs. Afterwards the wings were clipped and the flies recovered at 25 °C for 12 hrs before the test.

4. Conclusions

VirtualFlyBrain.org   a   b

The neuropil regions that result to be mostly marked by CD8-GFP expression are highlighted in a and in b: Saddle, Vest, Superior medial protocerebrum (a), and the Protocerebral bridge (b).

c´   c´´   c´´´

This scheme of the Stinger-GFP expression pattern reveal mostly a *dFoxP*-isoB clustered expression in the cortex, where we can identify four major bilateral clusters and two central ones (c´ and c´´´, green dots). The red arrowheads in c´ and c´´ indicate the **dorsal cluster neurons**, with projections spanning the entire central brain.

From the behavioral data we can extrapolate four major conclusions:

- comparing the two different set-ups (Buridan´s paradigm and Flight simulator), we notice that the heterozygous flies have two different outcomes: a dominant behavior in Buridan´s paradigm and a recessive one in the Flight simulator. This phenomenon is called **Differential Dominance**. Differential dominance is a situation in which the dominance effects at a pleiotropic locus vary between traits. The FoxP-iB heterozygous mutant flies in fact, despite of a WT learning ability, have a significant change in activity measurements.

- when we compare instead the results obtained from only one set-up (Buridan´s paradigm), we observe a clear phenomenon of **Overdominance**, which is a condition in genetics where the phenotype of the heterozygous lies outside the phenotypical range of both homozygous parents, and this can be seen in all of the temporal measures performed in Buridan´s.

- Knocking out all FoxP isoforms does not seem to have a stronger effect on general locomotor behaviour if compared to knocking out only isoform B.

- If FoxP is knocked out only in **motorneurons** we can already see a phenotype, suggesting that motorneurons are heavily involved. However, if FoxP is removed in every neuron exclusively during **adulthood** (or pupal stage), no effect is observed, suggesting a developmental function of this transcription factor.

5. References

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