Drosophila melanogaster as a model to study WNT pathway alteration in Cornelia de Lange Syndrome

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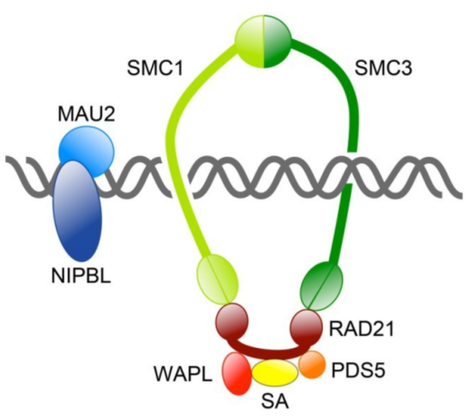
INTRODUCTION AND AIM

The cohesin complex is formed by a multi-subunit core and their associated regulatory proteins. Genetic variants within components of the cohesin complex (*NIPBL*, *SMC1A*, *SMC3*, *RAD21*, *HDAC8*) are believed to be responsible for the Cornelia de Lange Syndrome (CdLS), a multiple malformation syndrome affecting almost any organ and causing severe developmental delay. The cohesin complex has a canonical role in cell division and a non-canonical role in gene expression regulation. "Cohesinopathies" seem to be caused by dysregulation of specific developmental pathways downstream of mutations in cohesin components, and canonical WNT pathway is considered to be the most interesting in this process.

In this study, we will validate the importance of canonical WNT pathway in CdLS pathogenesis, using published *D. melanogaster* CdLS models. At the same time, we will exploit *D. melanogaster* for screening WNT-activator compounds (eg. LiCl, BIO, CHIR99021, Deoxycholic acid, IQ1).



Drosophila melanogaster

Drosophila melanogaster has been heavily used in research in genetics and is a common model organism in developmental biology. Therefore, we chose to use *D. melanogaster* in this study because it is an animal model that provides fast and relative inexpensive background to study CdLS and a handling good model for *in vivo* chemical screening. In literature many *Drosophila* models for CdLS have been described with mutations in cohesin complex genes. These models could provide new data in a different species rather than *D. rerio* or fibroblast cells from affected patients (Pistocchi et al., 2013; Fazio et al., 2016).

nipped-B (NIPBL)

nipped-B in *D. melanogaster* is the ortholog of the human *NIPBL* gene. Nipped-B interacts with Mau2 to load the cohesin ring complex onto chromosomes. Nipped-B and cohesin participate in transcriptional regulation and DNA repair.

We are testing the mutated loss-of-function allele ***nipped-B^{407}***.

nipped-B^{407} mutants are known to possess fewer cells with a smaller size in the adult stage, therefore these mutants' weight is lighter (Wu et al., 2015).

We confirm that mutated flies weight about 5% less than controls.

	Common food	Food added with LiCl
yw ♂	0,7440 mg	*not enough flies*
yw ♀	1,2360 mg	1,2222 mg
nipped-B ♂	0,7141 mg	*not enough flies*
nipped-B ♀	1,1780 mg	1,1571 mg

hdac3 (HDAC8)

hdac3 in *D. melanogaster* is the ortholog of the human *HDAC8* gene. Hdac3 is a histone deacetylase involved in chromatin silencing, gene transcriptional regulation and may be involved in the deacetylation of Smc3 in the cohesin complex.

We are testing 2 different alleles:
> ***hdac3^{N}*** (EMS mutation: removal of *hdac3* catalytic domain).
> ***hdac3^{6C}*** (Imprecise excision of a p-element: removal of about two-thirds of the *hdac3* coding region).

The depletion of *hdac3* in the fat body results in a reduction in body size (Lv et al., 2012).

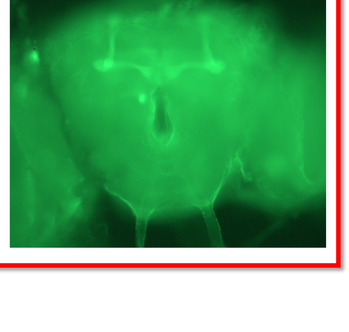
Working Flow

Every mutant stock was outcrossed with a *yw* strain in order to obtain the same genetical background. Flies were weighted to assess the differences in the body weight between mutants and *yw* strain. Then both strains, controls and mutated flies, were grown upon food added with a WNT activator (eg. LiCl) and after the enclosure of the pupae, adults were weighted again in order to discover if the mutated flies have gained weight with a phenotype improvement.
Mutant flies for *nipped-B* have been fed with LiCl at different concentrations (50mM, 100mM, 200mM and 500mM) and we found that the higher tolerated dose is 200mM since flies died when exposed to the 500mM dose.
Treatment with LiCl (50mM) did not improve mutants phenotype (see table above), although it might be related to the small difference in weight between mutant and wild-type animals.

LiCl 50mM ✓
LiCl 100mM ✓
LiCl 200mM ✓
LiCl 500mM ✗

Future direction

The next step is to find the best working dose for each drug we plan to add to the food. All drugs are well-known WNT canonical pathway activators: LiCl, BIO (Sato et al., 2004), CHIR99021, Deoxycholic acid (Pai et al., 2004), IQ1 (Miyabayashi et al., 2007). Since flies weight assay does not seem ideal for high-throughput screening, we now plan to assess other assays to investigate the phenotype, such as analyzing mushroom bodies, a brain structure known to be abnormal in nipped-B flies (Wu et al., 2015).



There is no conflict of interest to be disclosed