

Modulating the WNT pathway in *Drosophila* models of Cornelia de Lange Syndrome

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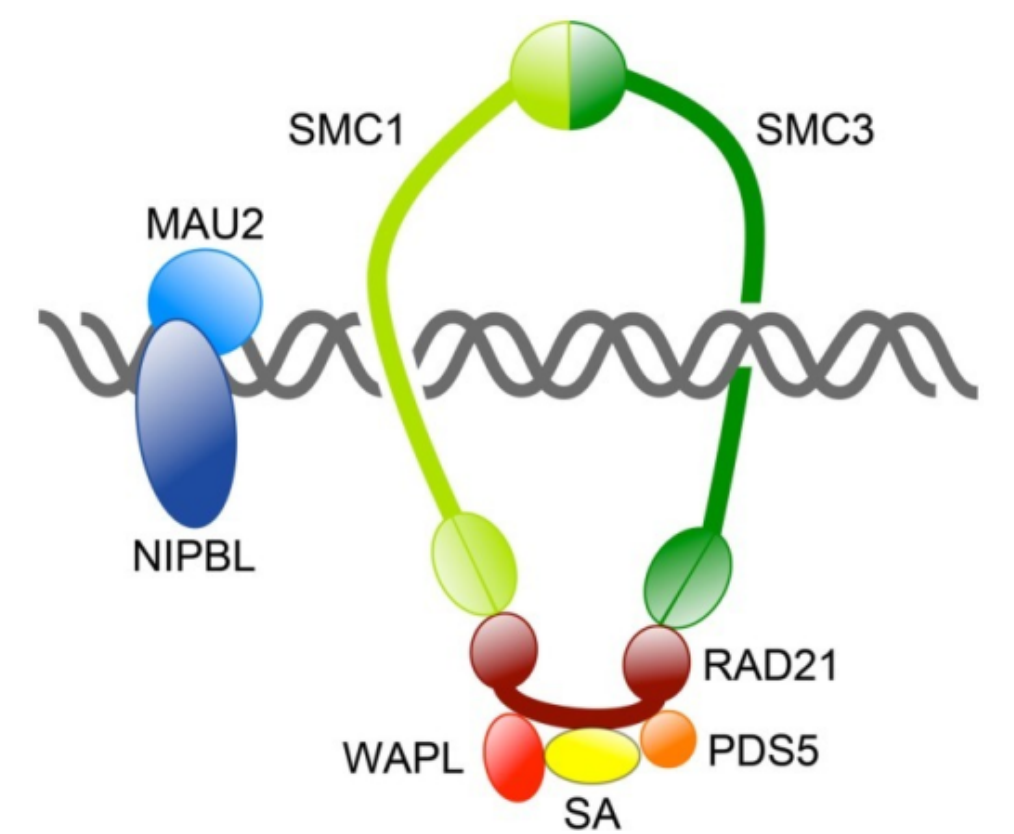
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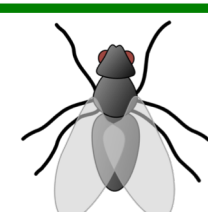
INTRODUCTION AND AIM



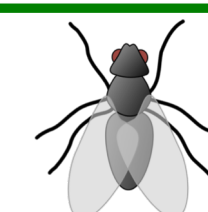
The cohesin complex is formed by a multi-subunit core and their associated regulatory proteins. Genetic variants within components of the cohesin complex (*NIPBL*, *SMC1A*, *SMC3*, *RAD21*, *HDAC8*) are believed to be responsible for the Cornelia de Lange Syndrome (CdLS), a multiple malformation syndrome 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).

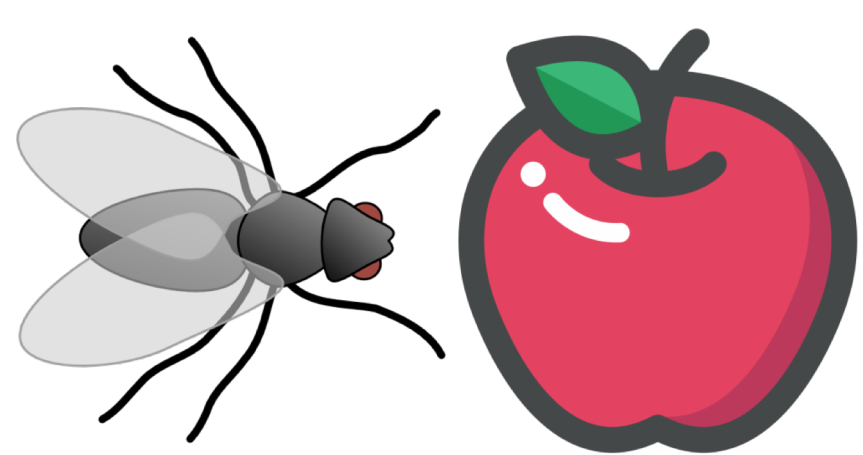


Working Flow

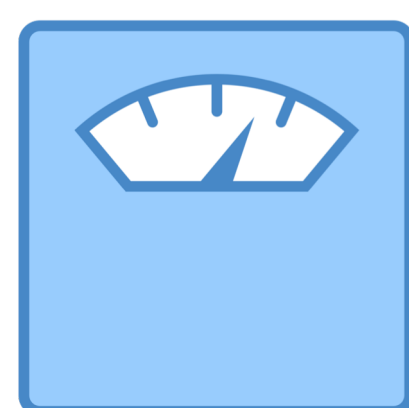


Every mutant stock was outcrossed with a yw strain in order to obtain the same genetical background. Flies were weighted to assess the differences in the body weight between mutants and yw strain. Then both strains, controls and mutated flies, were grown upon food added with a WNT activator (eg. LiCl) 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.

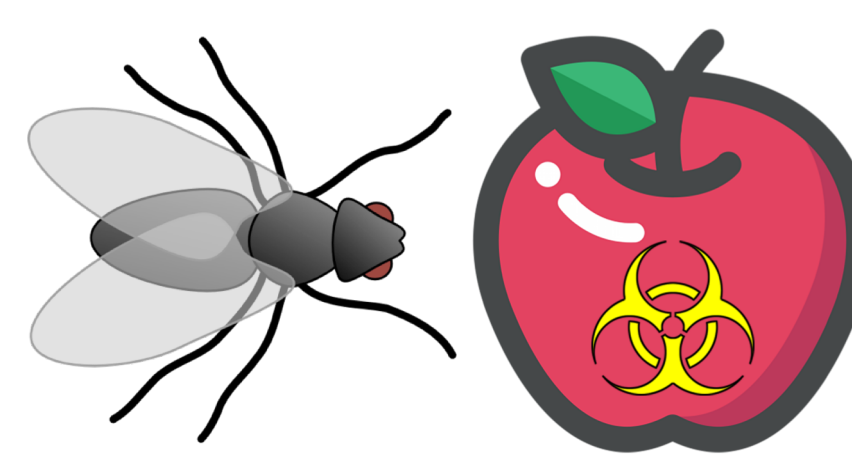
Mutant flies for *nipped-B* have already been fed with LiCl at a low concentration of (50mM) in fact, no improvements in the phenotype were observed (see table above). We used this low dose to assess that the protocol was set up correctly and therefore, could be used to treat flies without side effect or lethality.



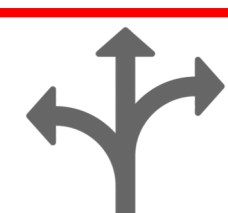
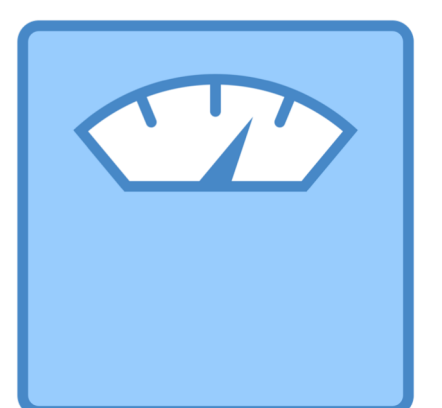
Common Food



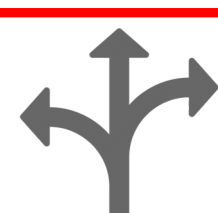
Vs



Food with drug



Future direction



The next step is to find the best working dose for every drug we plan to add to the food. All the 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).

At a later time we want to take advantage of western blot technique to assess whether Smc3 is a direct target of Hdac3 enzymatic activity in *Drosophila* as already demonstrated in other species (human, mouse, zebrafish). Moreover, we want to investigate the WNT canonical pathway dysregulation by detecting important proteins involved in this process, such as cyclin D1 and activated β-catenin.