A Thesis submitted

In Partial Fulfillment of the requirements

for The Degree of

Master of Science

By

M. Adishree

To

National Institute of Science Education and Research

Jatani Campus, Padanpur, Khordha

Bhubaneswar 752050

Odisha, India.

DECLARATION

I hereby declare that I am the sole author of this thesis in partial fulfillment of the requirements for a postgraduate degree from National Institute of Science Education and Research (NISER). I authorize NISER to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Adishree

Signature of the Student

Date: 01.06.2020

The thesis work reported in the thesis was carried out under my supervision, in the school of biological sciences, at NISER, Bhubaneswar, India.

Signature of Thesis Supervisor

School : SBS

Date :

ACKNOWLEDGMENT

Firstly, I would like to express my sincere gratitude to my supervisor, Dr. Renjith Mathew for giving me the opportunity to work on this exciting and innovative project.

I would like to extend my gratitude to Dr. Sanjita for her able guidance, advice and suggestions.

My sincere thanks to my mentor, Rojalin Pradhan for her continuous support, patience, motivation, and immense knowledge. I thank my fellow lab mates Saumya, Deepsikha, Dipika, Dr. Supriya, Nivedita, Prabeer for the stimulating discussions, for always lending a helping hand, and for all the fun we have had in the last two years. Without their love and support, this project would not have been the same.

I would also like to thank the members of my advisory committee: Dr. Debasmita, Dr. Pankaj, Dr. Manjusha, for their comments and suggestions.

Lastly, I would like to thank my friends and family for their endless love and support in the long run of my life.

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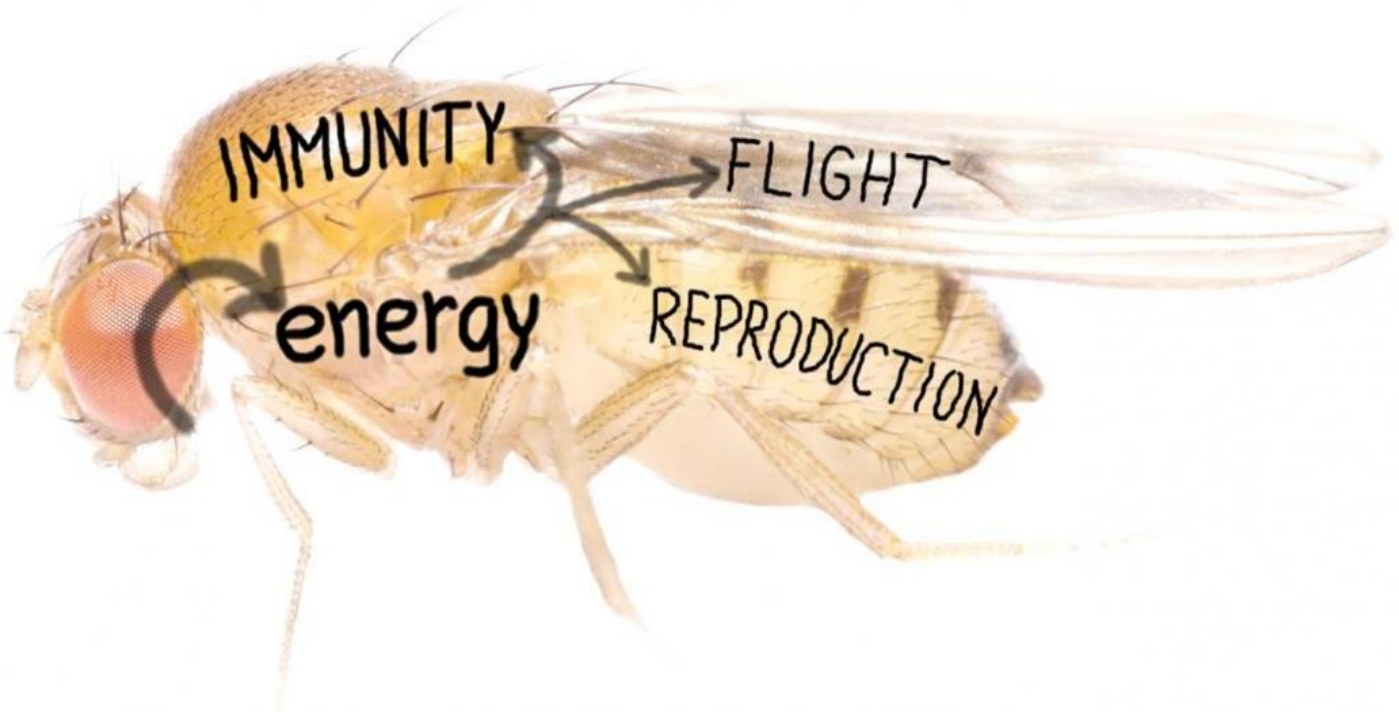
IMMUNITY
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UNIT I: ABIOTIC STRESSORS & ORGANISMAL RESPONSE

ABBREVIATIONS & FORMULAE USED

NF-κB : Nuclear factor kappa B

Imd pathway: Immune deficiency pathway

ROS: reactive oxygen species

VEGF: Vascular endothelial growth factor

PI3K: Phosphoinositide 3-kinase

mTOR: Mammalian target of Rapamycin

ERK: Extracellular-signal-regulated kinase

$CoCl_2$: Cobalt chloride

Mw: molecular weight

1M= 1 **molal** solution

Mm: millimolar

M1V1=M2V2 where,

M1= concentration of the concentrated solution in molarity (moles/Litres)

V1= volume of the concentrated solution,

M2= concentration of the diluted solution (after addition of more solvent) in molarity,

V2 = volume of the diluted solution.

BG4-PIP2-GFP: *breathless* Gal4-Phosphatidylinositol 4,5-bisphosphate2-green fluorescent protein

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AIM: To study hypoxia induced immune responses in *Drosophila melanogaster*.

INTRODUCTION

Hypoxia is characterized as the reduction or lack of oxygen in cells, organs, or tissues. It is an important stimulus involved in both physiological and pathological processes. This project mainly focuses on the impact of hypoxia on the immune system and the interdependence of hypoxic and innate immune responses.

The fruit fly *Drosophila melanogaster* is highly resistant to oxygen starvation as the larvae compete with microorganisms for limited amounts of oxygen in its normal habitat, and thus serve as a good model system to study physiological, developmental, and cellular adaptations to hypoxia.

Also, the lack of an adaptive immune system, makes *D. melanogaster* a powerful model system, for studying the aspects of innate immune system that otherwise might be obscured by the presence of adaptive immune system.

LITERATURE REVIEW

The response against hypoxia is mediated by the family of hypoxia-inducible factor (HIF) transcription factors, present in cells & tissues. Hypoxia-inducible factor-1 (HIF-1), is a multi-subunit protein that directs the transcription of hypoxia response elements (HRE), & is composed of 2 basic HLH proteins: HIF-1α and HIF-1β. A similar hypoxia responsive system, homologous to mammalian HIF has been reported in the fruit fly, where Tango and Sima proteins are the Drosophila homologues of mammalian HIF-β and HIF-α units, respectively.

During normal oxygen levels, post translational hydroxylation of the proline residue in the oxygen dependent degradation domain (ODDD), by proline hydroxylases, allows Von Hippel-Lindau protein (pVHL), a ubiquitin complex protein, to bind to the HIF-α unit. Binding of pVHL to HIF-α, marks it for ubiquitination and rapid degradation.

During hypoxic conditions, proline hydroxylases are unable to hydroxylate the proline residue required for the interaction between pVHL and HIF-α unit, thereby preventing the degradation of the same. The HIF-α unit binds to the β-unit and drives the transcription of HREs.

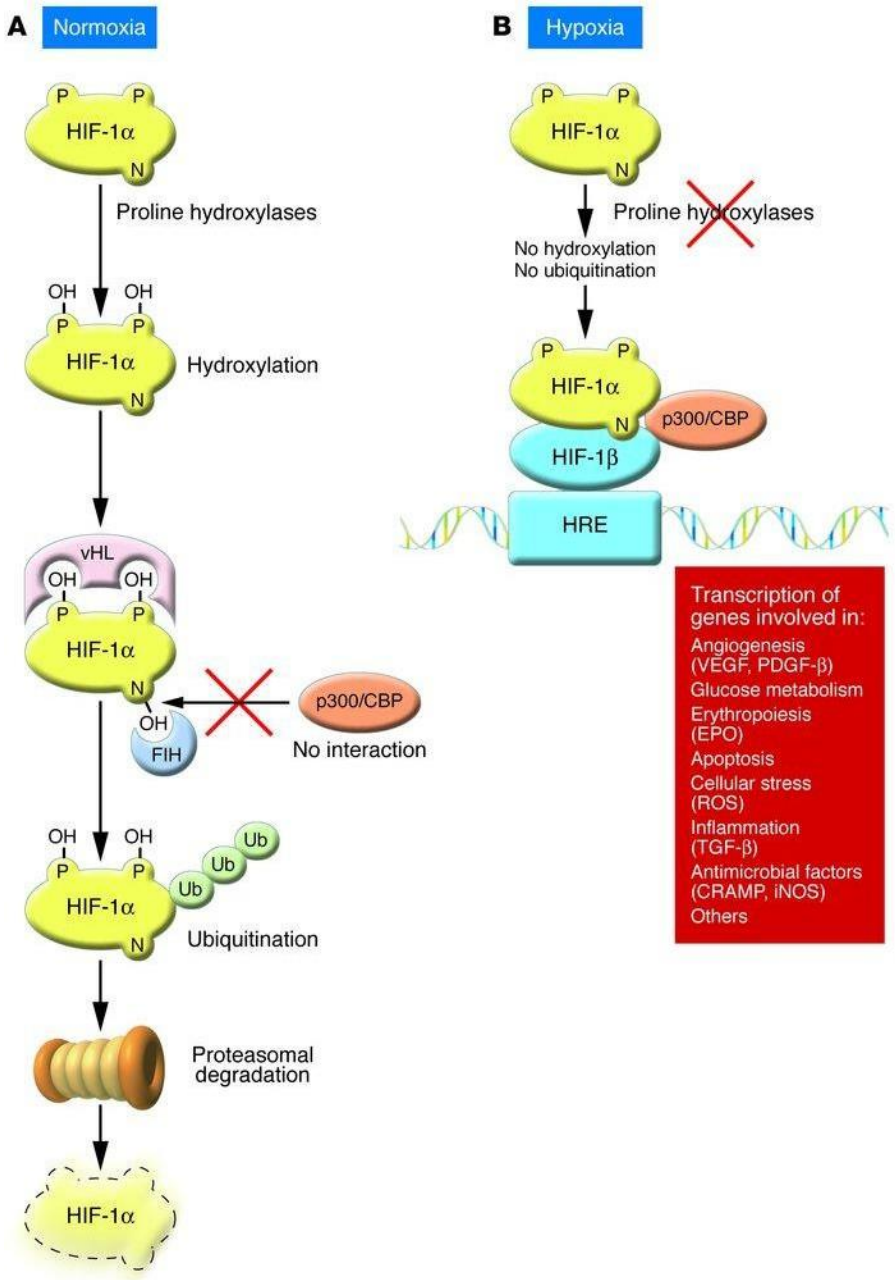

Figure 1: Action of hypoxia inducible factor (HIF-1) in Normal vs Hypoxic conditions. (Kol A. Zarember, Harry L. Malech, 2005)

Cobalt mimics hypoxia by directly binding to the ODDD of the HIF-α, inhibiting VHL binding to the HIF-α, thus impeding the degradation of HIF-α.

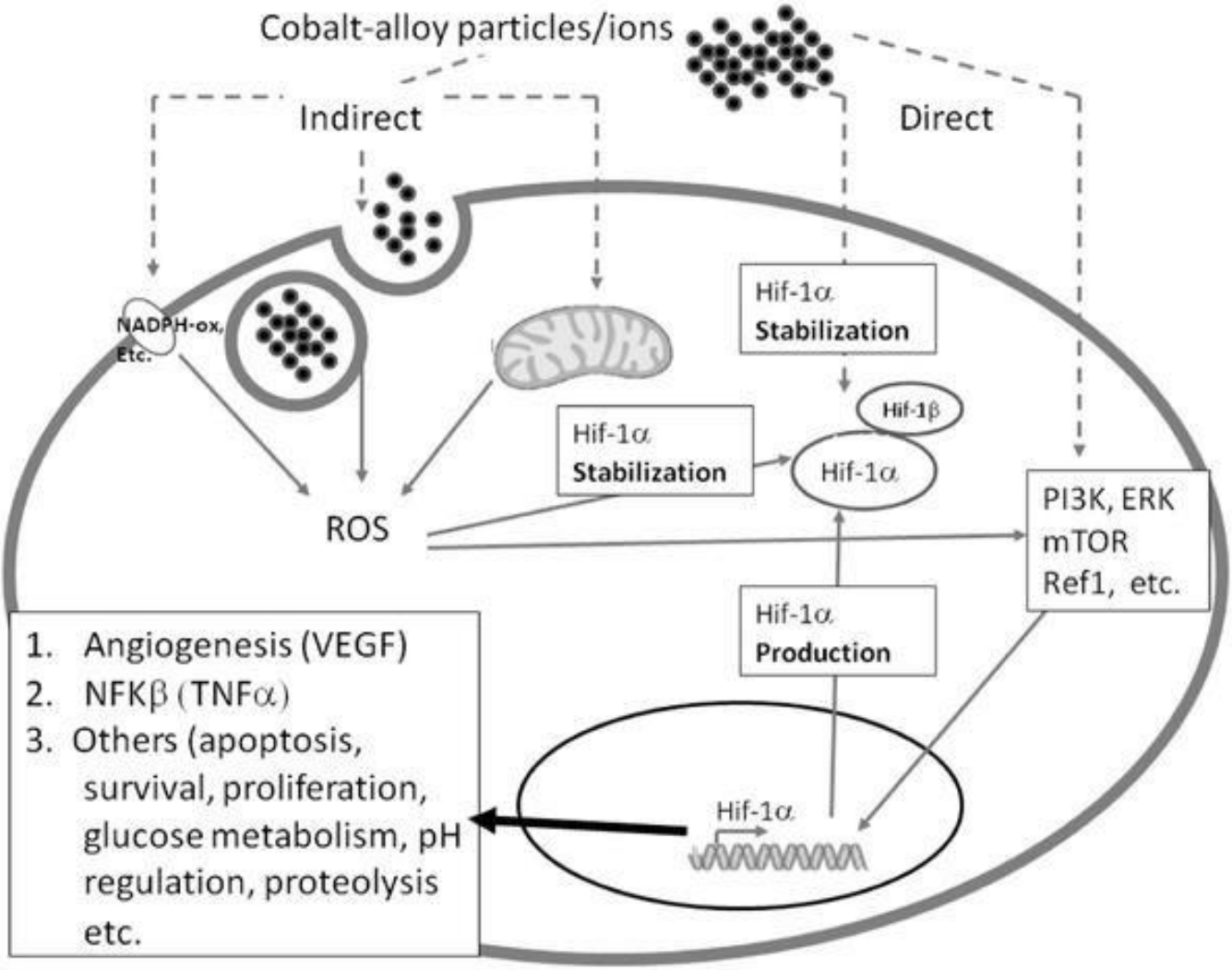

Figure 2: Direct and indirect pathways of cobalt induced hypoxic response

(Samelko, Lauryn & Caicedo, *et al* e67127. 10.1371/journal.pone.0067127)

Cobalt (Co) is needed as a trace element in almost all living forms to meet a number of metabolic functions. Though less prevalent in metalloenzymes than other abundant metals like Copper (Cu) or Zinc (Zn), Cobalt is a vital co-factor in vitamin-B12 enzymes.

Its deficiency can lead to increased risk of developmental anomalies and growth failures in neonates [2]. However, immoderate amount of Cobalt can be toxic. Whilst the definite mechanism(s) of Cobalt related toxicity is not yet established, several prospective mechanisms have been proposed.

It has been reported that, particularly, the iron-sulphur proteins are significantly affected by cobalt treatment. Studies show that Cobalt related toxicity is associated to its impact on Iron metabolism, primarily on [Iron-Sulphur] cluster assembly process.

Cobalt can induce hypoxia indirectly, by its capacity to form reactive oxygen species (ROS), which tend to stabilize the HIF-1, known to govern transcription of HREs.

- **Cellular & developmental adaptations to hypoxia**

- Hypoxia is known to promote extra sprouting of tracheal branches in Drosophila.
- In non-tracheal cells, Sima accumulation induces Branchless (Btl), a homolog of mammalian fibroblast growth factor (FGF).
- In tracheal cells, Sima induces the Breathless Branch(Btl), a receptor tyrosine kinase, mammalian FGF receptor (FGFR) homolog .
- Bnl binds to btl and predicts the direction of tracheal cell migration and consequent branch extension.

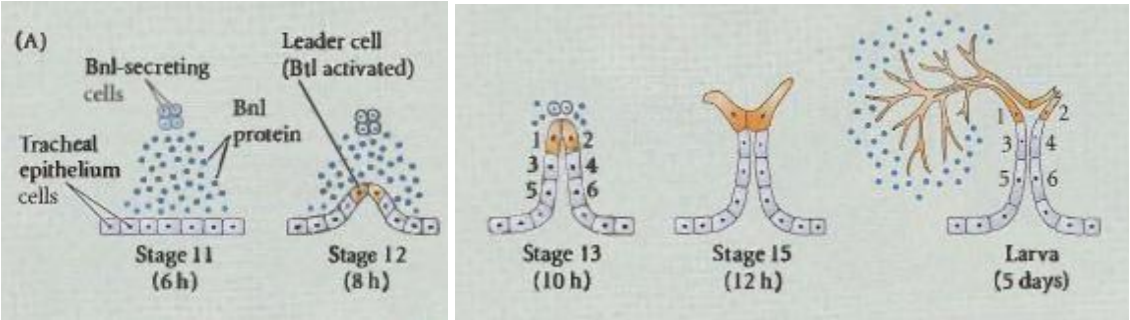

Figure 3: Tracheal development in *Drosophila melanogaster*. (A) Pictorial representation of budding dorsal tracheal branch from tracheal epithelium. Cells in the vicinity, release Branchless protein (Bnl; represented by blue dots), which then activates Breathless protein (Btl; represented by yellow colouration of cells). (B) The activated Breathless protein stimulates migration of the leader cells & tube formation; the dorsal branch cells are numbered 1 through 6. Bnl also induces unicellular secondary branches. *(GILBERT, 11th ed)*

- **<u>Hypoxia induced immune responses</u>.**

It has been reported that hypoxia activates NF-κB expression independent of the developmental stage.

- Two distinct NF-κB pathways namely, IMD & Toll Pathways, reportedly regulate the formation of antimicrobial peptides (AMPs) by recruiting the functions of respective NF-κB transcription factors.

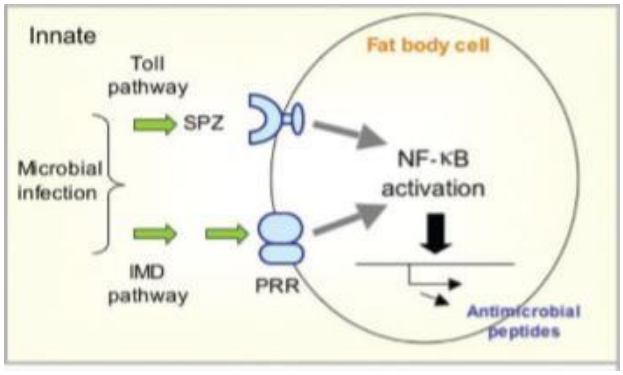

Figure 4: NF-κB pathways

- These AMPs are secreted from cells of the fat bodies into the hemolymph.
- The IMD pathway is responsible for resistance to Gram-negative bacterial pathogenesis, whereas the Toll pathway defends hosts against fungal and Gram-positive bacterial infections.

		Downstream genes	Anti-microbial peptides
Toll pathway	Toll1 receptor	**Dorsal** and **Dif**	**Drosomycin and Metchnikowin**
Imd pathway	receptor	**Relish**	**Attacin, Cecropins, Defensin, Diptericin, & Drosocin**
RNAi machinery		**Dicer-2, Argonaute-2**	

LACUNAE:

- Which all tissues are involved in the immune response?
- How exactly hypoxia induces immune response?
- Which immune response pathway(s) is/are activated?

OBJECTIVES:

(1) To establish a hypoxia model, chemically, using cobalt chloride.

(2) To check for immune responses induced by hypoxia.

(3) To determine the immune pathway candidates.

(4) To track down the tissues involved in the immune response pathway.

(5) To determine the link between hypoxia and immune response.

METHODOLOGY

(1) To establish a chemical hypoxia model using cobalt chloride.

Media preparation and Cobalt chloride stock

100ml fresh media was prepared and autoclaved at 121°C for 15 minutes:

Ingredients	Amount
+ water	100ml
yeast	1.5g
agar	0.9g
sugar	4g
glucose	2g
Corn flour	8g

Meanwhile, $CoCl_2$ stock was prepared:

Mw of $CoCl_2$ = 129.839g so, 1M= 129.839g in 1L

0.5M= 64.9g in 1L

For 5ml 0.5M $CoCl_2$, 0.3g $CoCl_2$ added in 5ml distilled water.

Media allowed to cool down. Then, antifungal reagents added:

Ingredients	Amount (in µl)
Propionic acid	400
Orthophosphoric acid	600
TEGO (1g methyl paraben in 5ml ethanol)	625

**Out of 100ml media, ~20ml poured into autoclaved vials that is to be used as control.

Of remaining 80ml,

(i) For a final $CoCl_2$ concentration of 0.5mM:

M1V1=M2V2
500mM * V1 = 0.5mM * 80ml
V1 = 80 µl

(ii) For a final $CoCl_2$ concentration of 1.5mM:

M1V1=M2V2
500mM * V1 = 1.5mM * 60ml
V1 = 120 µl

(iii) For a final $CoCl_2$ concentration of 3.5mM:

M1V1=M2V2
500mM * V1 = 3.5mM * 40ml
V1 = 160 µl

(iv) For a final $CoCl_2$ concentration of 8.5mM:

M1V1=M2V2
500mM * V1 = 8.5mM * 20ml
V1 = 200 µl

**similar procedure followed for all the experiments.

All the vials labelled properly and kept at room temperature overnight. Then used the very next day. For all the experiments, food was prepared fresh just a day before.

Fly strain, mating, experimental design

The experiments were performed with BG4-PIP2-GFP drosophila larvae. Flies were kept in the 25°C light cycle incubator.

Mating was set-up in cages with 30-40 flies for 4hours, then flies transferred to separate vials. The cages with eggs were left undisturbed in 25°C light cycle incubator, for next 24hours.

Hypoxic incubations started with larvae after hatching. Exactly 24hours later, newly hatched larvae transferred to separate food vials of different $CoCl_2$ concentrations.

Control groups were raised in fresh media without added $CoCl_2$. For the first set of experiment, $CoCl_2$ was used at final concentrations of 1mM and 50mM as per the decided range **(Exp-1)**. Then, for the next set of experiment, $CoCl_2$ was used at final concentrations of 0.5mM, 1.5mM, 3.5mM and 8.5mM **(Exp-2)**.

To replicate and reproduce the results, a final set of experiment was done with $CoCl_2$ concentrations, 0.25mM, 0.5mM, 1mM and 2mM **(Exp-3)**.

After transferring the larvae, the vials were kept in 25°C light cycle incubator and observations taken every day till all the larvae eclose.

Dosage range was narrowed to 0.25mM-10mM. Survival, growth and development was recorded every day.

Evaluation of hypoxia

HIF1-α antibodies were ordered from Abcam for western blot.

Observations and results

Exp-1: Flies were monitored daily for survival and growth using two concentrations of $CoCl_2$.

conditions	control	1mM $CoCl_2$	50mM $CoCl_2$
results	Normal development, 90% survival.	developmental retardation, with 90% survival.	Increased mortality, no survivors.

Table 1: Results showing survival percentage in control and $CoCl_2$ treated larvae.

Exp-2: Different concentrations of $CoCl_2$ was used and various parameters measured.

I. Survival profile

$CoCl_2$ concentration (in mM)	0	0.5	1.5	3.5	8.5
% survival	86%	90%	70%	-	-

Table 2: survival percentage in control and different $CoCl_2$ concentrations. In the higher doses a (3.5mM and 8.5mM) of $CoCl_2$, none of the larvae survived.

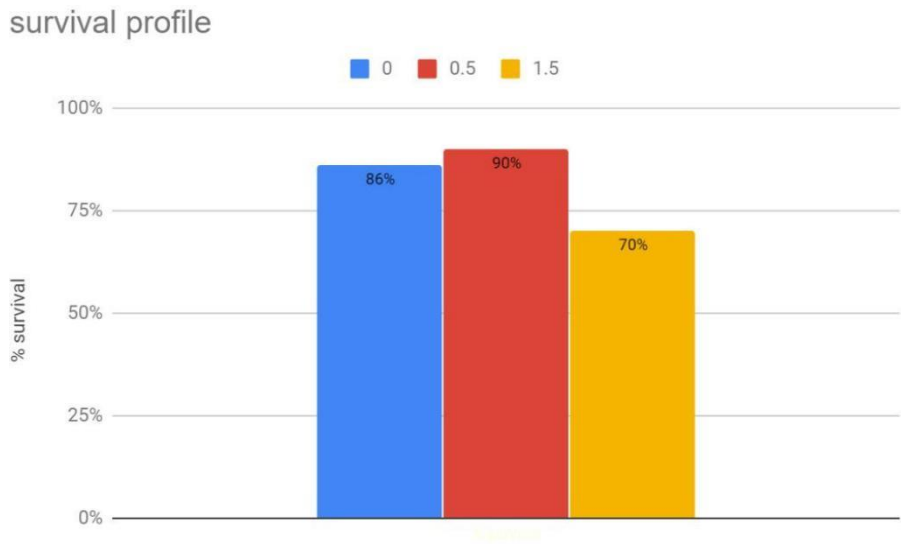

Figure 5: While the survival percentage is almost similar in control and lesser $CoCl_2$ concentration, as much as 0.5mM, the mortality rate is higher in higher $CoCl_2$ concentrations.

II. Pupal development: the larvae were monitored every day and the number of pupae counted in order to ascertain pupation rates.

$CoCl_2$ concentration (in mM)	0mM (control)	0.5mM	1.5mM
	(no. of pupae)	**(no. of pupae)**	**(no. of pupae)**
DPH			
6th	1	0	0
7th	8	0	2
8th	2	5	4
9th	2	3	3
10th		7	4
11th		3	1
total	13	18	14

Table 3: development of larvae to pupa in days post hatching (DPH).

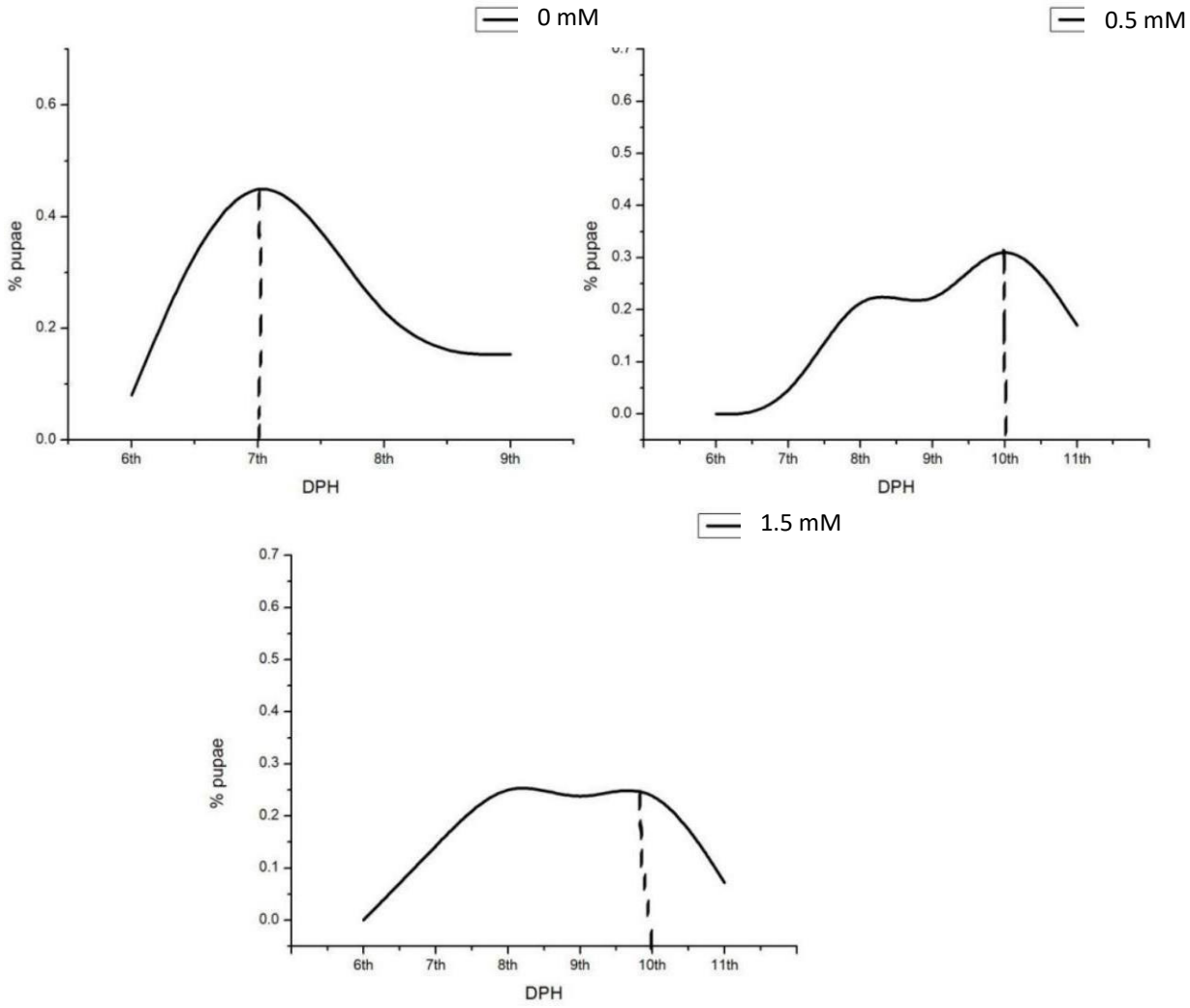

Figure 6: with an increase in $CoCl_2$ concentrations, there is a shift in the peak pupation time towards the right (represented by dotted lines) of the curves in case of $CoCl_2$ treated conditions as compared to the control. This indicates a delay in development.

III. pupal size

$CoCl_2$ concentration (in mM)	0	0.5	1.5
average pupa size (in mm)	2.8	~2.5	~2.5
standard deviation	0	0.4013050606	0.3977961265
p-value		0.004330794819 << 0.05	0.01863556672 <0.05

Table 4: for n=20, there is a significant reduction in the average pupal size in $CoCl_2$ treated conditions as compared to the control.

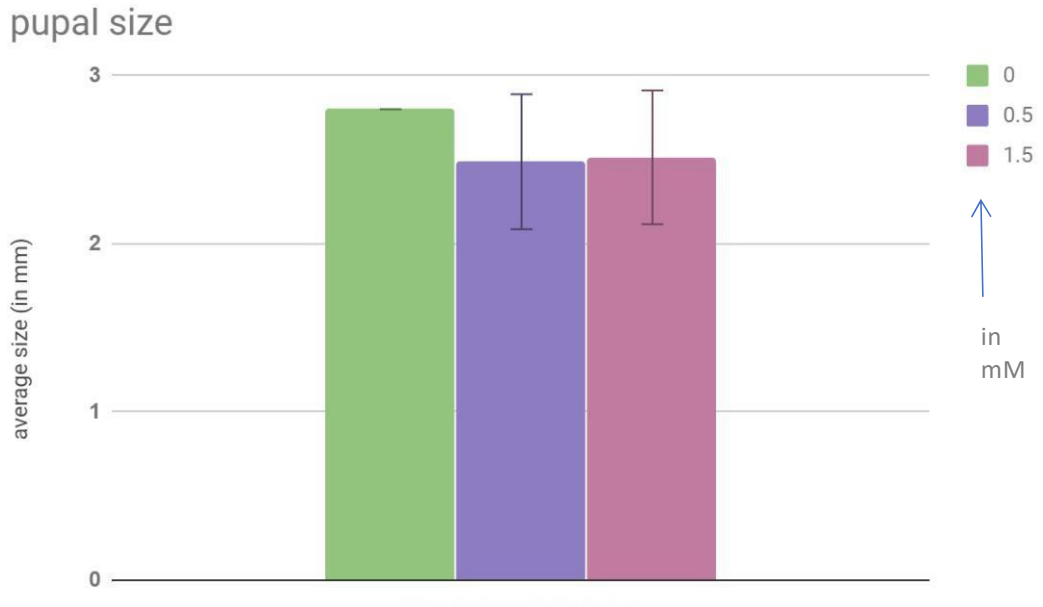

Figure 7: Significant reduction in the average pupal size.

Exp 3

I. Survival profile

$CoCl_2$ concentration (in mM)	0	0.25	0.5	1	2
% survival	80%	76%	64%	43%	0

Table 5: survival percentage in control and different $CoCl_2$ concentrations. None of the larvae survived 2mM $CoCl_2$ treatment, though they were seen climbing the walls, which is a classic stress response.

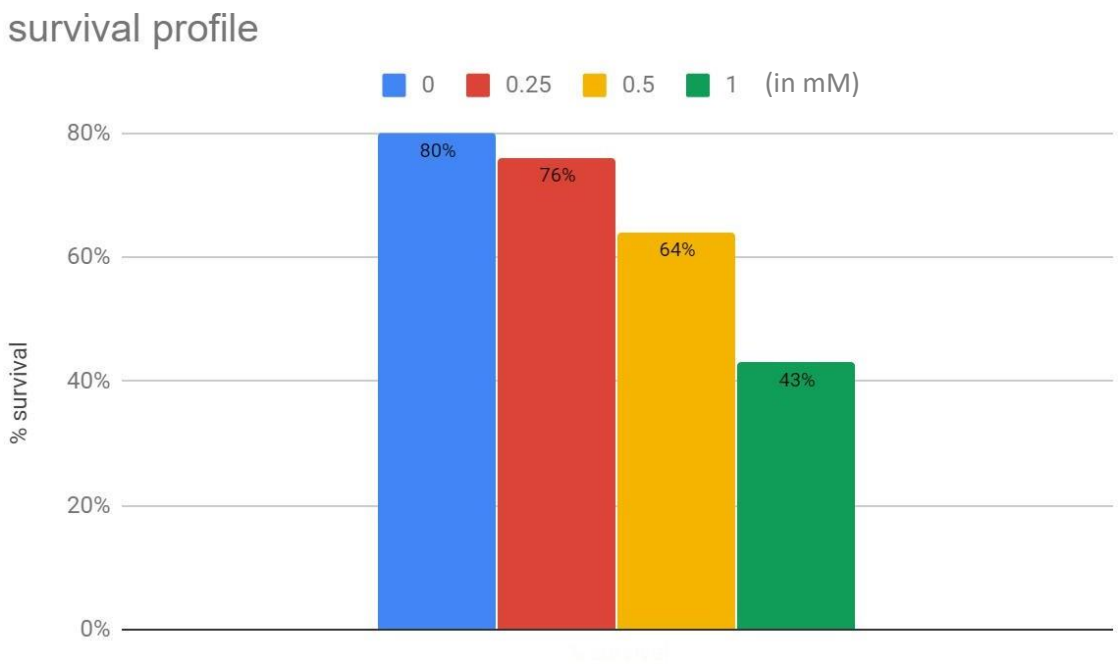

Figure 8: There is a gradual decrease in the percentage survival with increase in $CoCl_2$ concentrations.

ii. Pupal development

$CoCl_2$ concentration (in mM)	0	0.25	0.5	1
	no. of pupae	no. of pupae	no. of pupae	no. of pupae
DPH				
6th	6	0	0	0
7th	9	6	2	1
8th	12	5	7	3
9th	4	8	9	2
10th		13	5	7
11th		6	9	

Table 6: development of larvae to pupa in days post hatching (DPH).

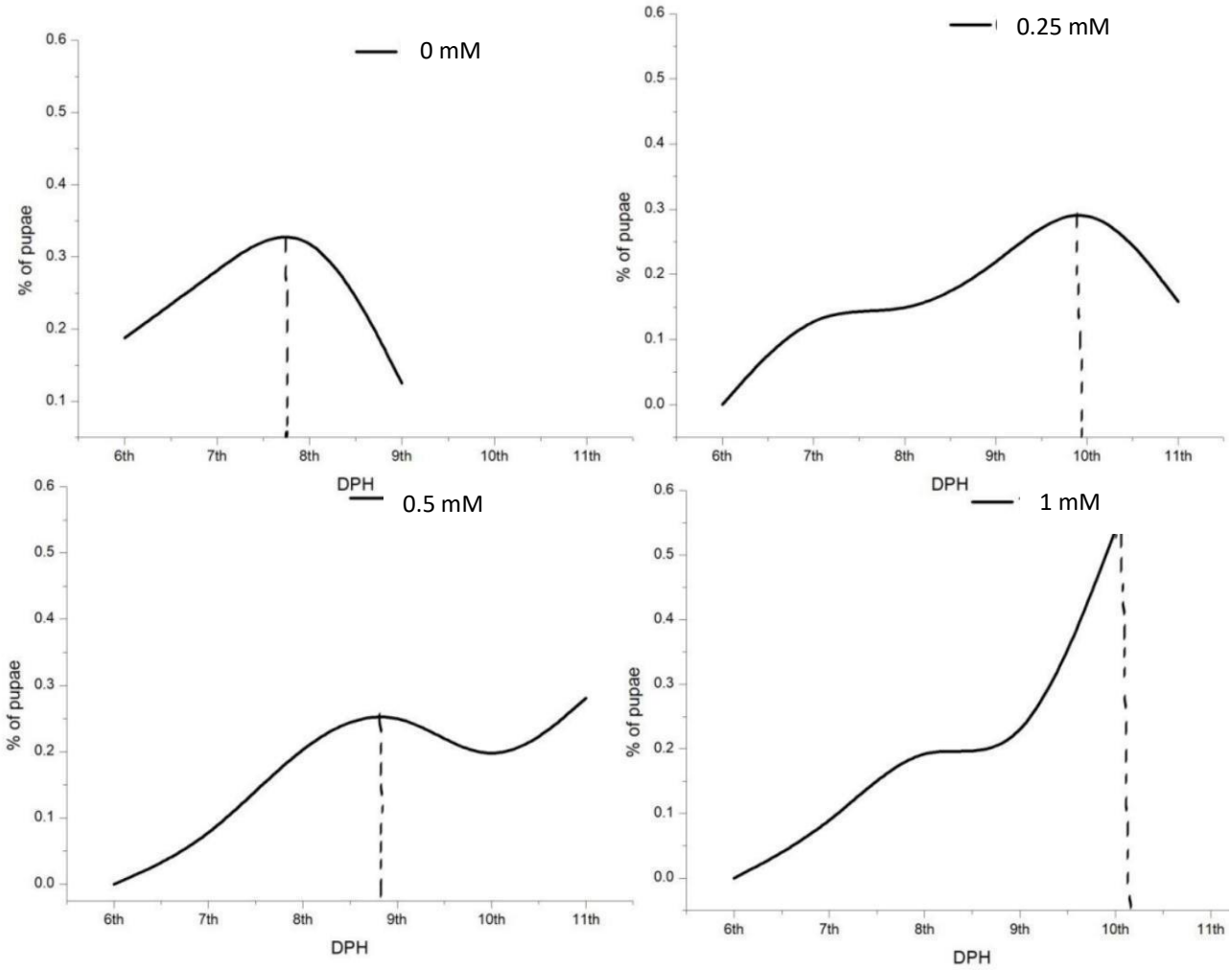

Figure 9: The peaks (represented in dotted lines) of the curves represent the days most of the larvae developed into pupae in control, 0.25mM, 0.5mM and 1mM $CoCl_2$ respectively. Shift in the peak position towards the right, indicate delay in development.

iii. Pupal size

CoCl2 concentration (in mM)	0	0.25	0.5	1
Average pupal size (in mm)	2.8	~2.6	2.6	~2.5
standard deviation	0	0.3676846939	0.3519530761	0.4050957468

Table 7: Average pupa size.

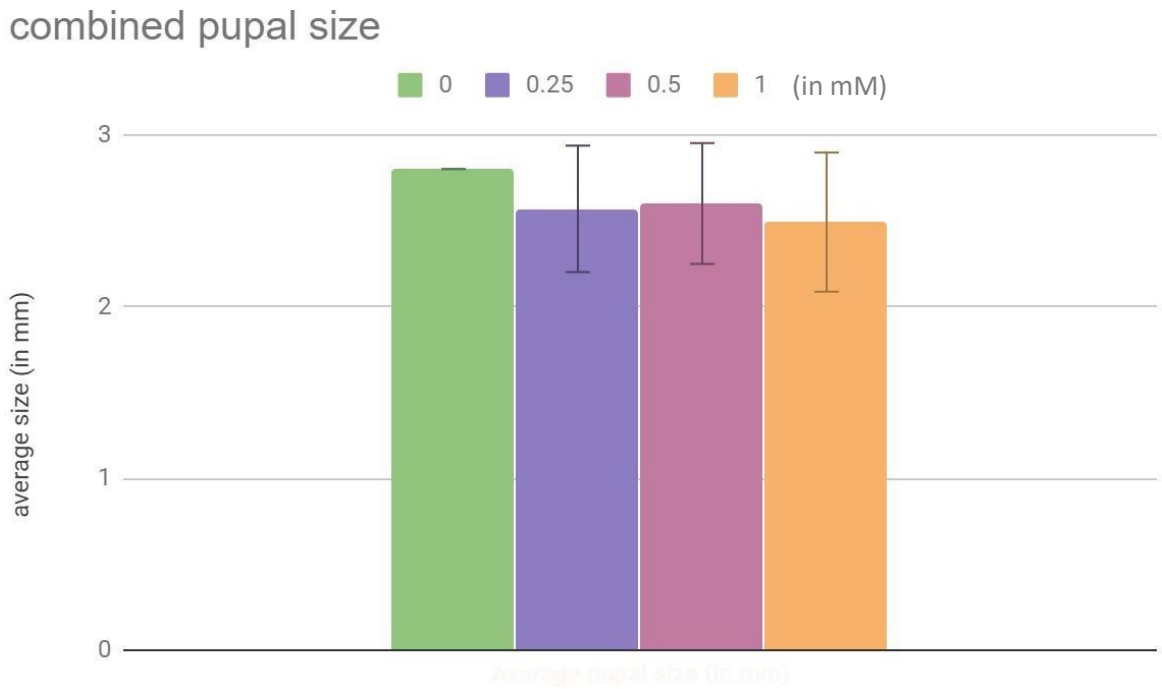

Figure 10: The average pupa size seems to be similar in different $CoCl_2$ conditions, but less as compared to the control.

Conclusion

1. 2mM $CoCl_2$ seems to be the critical concentration, below which larvae survive and above which all the larvae die within 48-72hours.
2. 1.5mM is the highest $CoCl_2$ concentration, with about 60-70% survival and showing the a phenotype.
3. Developmental retardation seen, maybe due to the $CoCl_2$ toxicity and not hypoxia. Western blot and qRT-PCR will confirm the hypoxia model.

Result

Due to inconsistent results, the project was terminated.

The inconsistency in results might be due to the following:

- Flawed mode of $CoCl_2$ administration.
- The full report on the after effects of $CoCl_2$ consumption on growth and development is still inconclusive.

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UNIT II:

TO CREATE A NOVEL TOOL FOR RNAi STUDIES IN *DROSOPHILA MELANOGASTER*

ABBREVIATIONS USED

RNA: Ribonucleic acid

miRNA: micro RNA

siRNA: small interfering RNA

RdRp: RNA dependent RNA polymerase

PCR: polymerized chain reaction

aPKC: atypical protein kinase C

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AIM: To generate and validate a recombinant fly line showing enhanced RNAi activity.

INTRODUCTION

RNA interference (RNAi), is a conserved biological response among various organisms, to dsRNA or shRNA. RNAi provides resistance to pathogens, and also governs the expression of protein coding genes. This post-transcriptional, sequence specific gene silencing mechanism, claims to revolutionize biology. With the advent of technology, its progresses & accuracy in gene targeting have enabled a myriad of now feasible applications, ranging from targeting casualties in crops, to bettering human health.

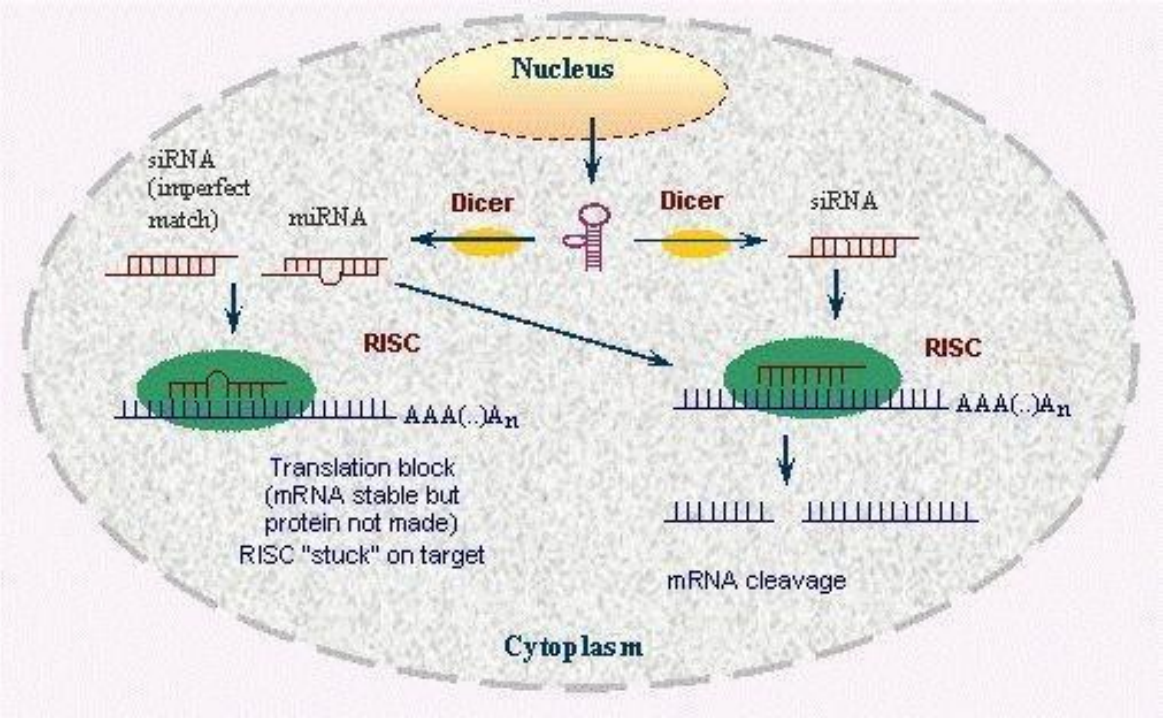


Figure 1: A simplified model of RNAi pathway. **[1]**

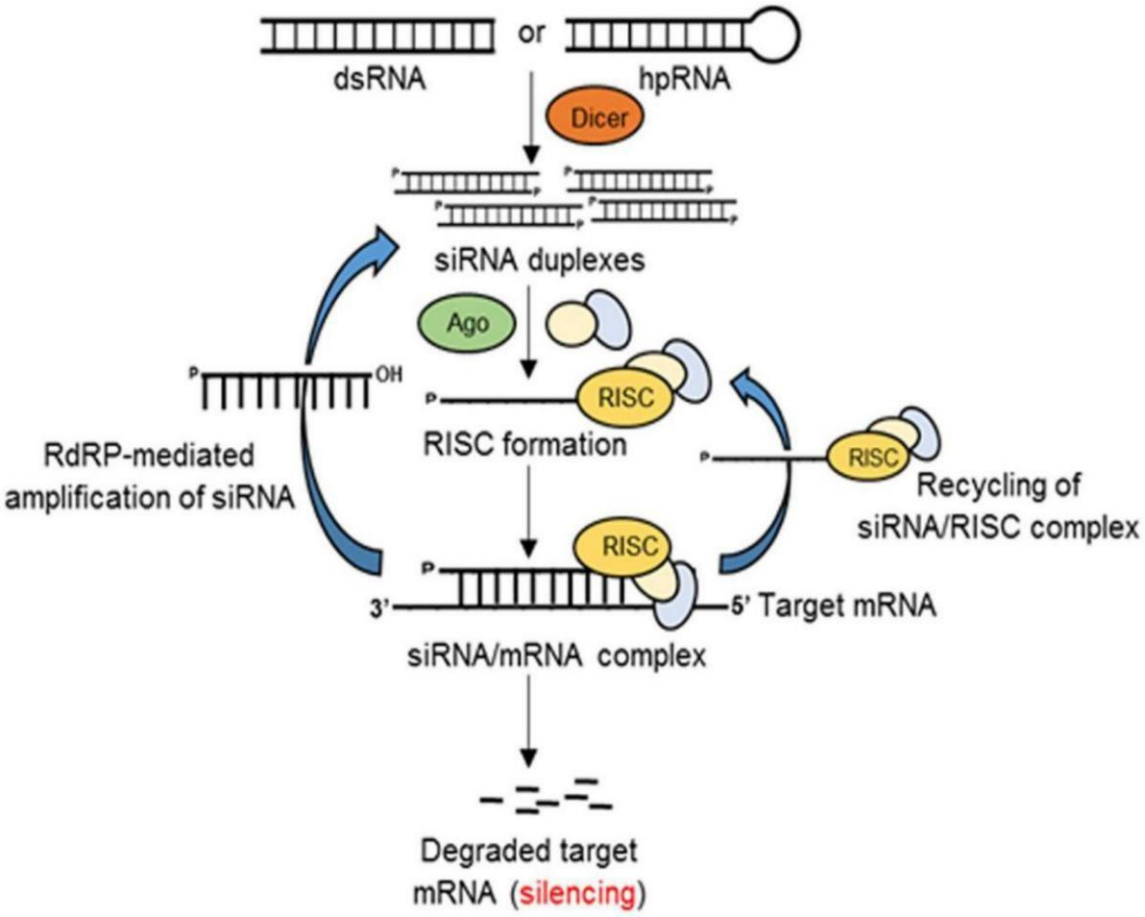


Figure 2: **Schematic of RNAi-mediated gene silencing in eukaryotes** [2]

The process of silencing [3]:

i. Fragmentation of ds RNAs into 21–25nt fragments of siRNAs in cytoplasm.

ii. catalysis by **Dicer** enzyme.

iii. Incorporation of siRNAs into the multi-protein silencing complex, all-together termed as RNA induced silencing complex (RISC).

iv. uncoiling of the siRNA duplex, followed by activation of the RISC.

v. Guide siRNA (antisense RNA) identifies the target mRNA (mRNA to be degraded), and then signals RISC for the endonucleolytic degradation of the same.

Dicer is classified among the RNase III endoribonucleases, that assists in generation of fragmented siRNAs from long double stranded RNAs, in a ATP-dependent process. Structurally, it is a ~220-kDa multi-modular protein.

RISC is a ribo-nucleoprotein complex that cleaves mRNAs through a sequence specific nuclease. RISC has a catalytic component, **Argonaute** (AGO), also an endonuclease that fragments the target mRNA strand complementary to their bound siRNA.

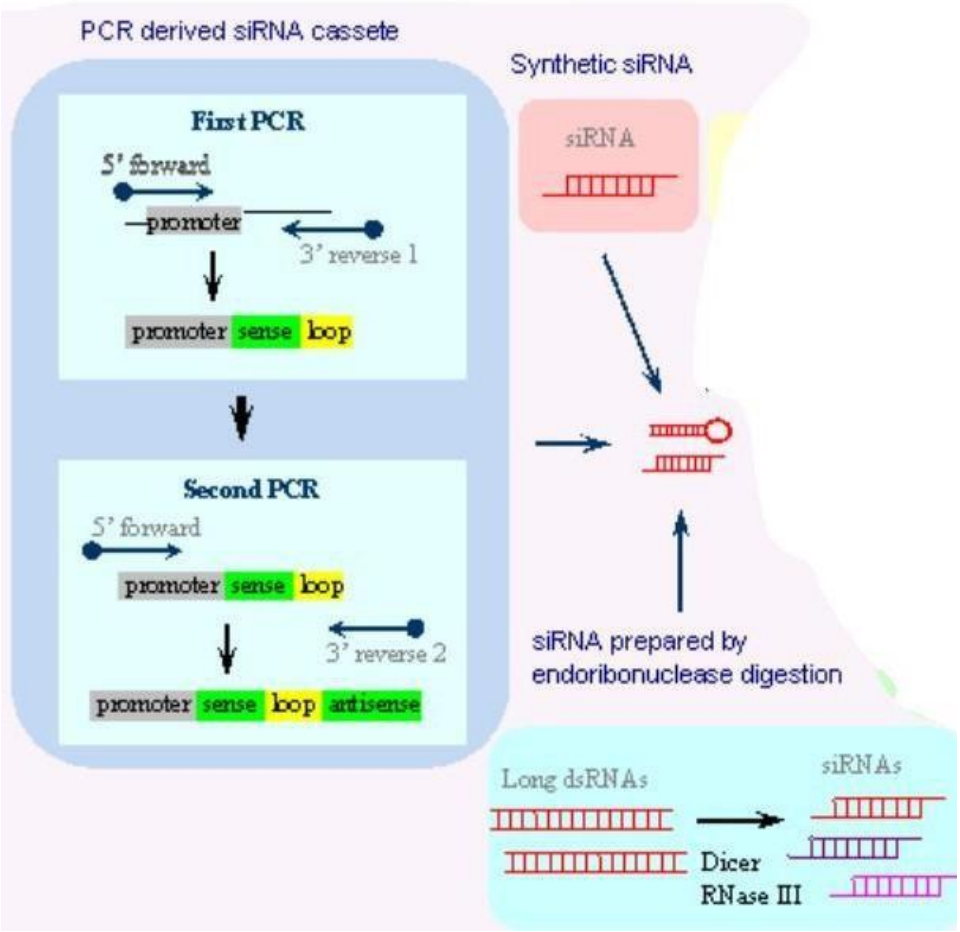


Figure 3: **RNAi in experiments & therapeutics: how it works**

Objective: Develop a Dicer fly line asserting enhanced RNAi activity.

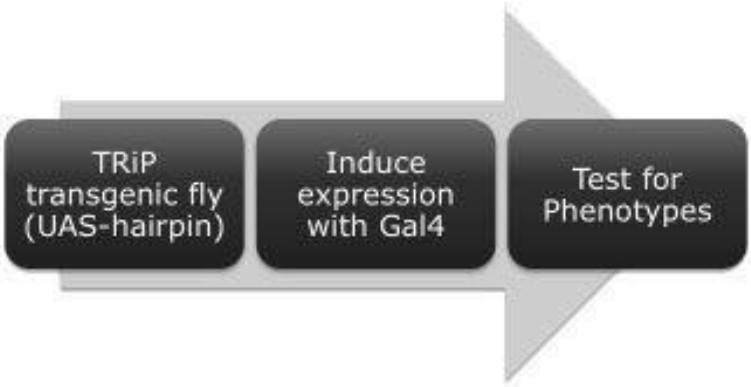


Figure 4: **Transgenic RNAi Project (TRiP) workflow** [4]

LITERATURE

Upstream activator sequences (UASs), present upstream of the core promoter, are DNA elements that bind to regulatory proteins (activators and repressors), and regulate transcription in vivo by helping or hindering transcription from the core promoter.

Gal4 is a eukaryotic activator, activates transcription of the galactose genes in the yeast *Saccharomyces cerevisiae*. These genes encode enzymes required for galactose metabolism, e.g. GAL1. When bound upstream of GAL1, in the presence of galactose, Gal4 activates transcription of the GAL1 gene 1000-fold. Gal4 does not exist endogenously in flies and does not act on any endogenous loci in the fly genome.

Inducing RNAi using the **UAS-GAL4 system**, spatial-temporal control over gene knockdown in adult fly tissues can be achieved:

- promoter or enhancer elements drive the expression of the eukaryotic transcription activator GAL4,
- once expressed, GAL4 binds a DNA response element, the upstream activating sequence (UAS), which precedes a gene fragment containing an inverted repeat that is exactly complementary to gene of interest.
- Expression of the inverted repeat drives the formation of dsRNA hairpins, or loops, that elicit a sequence-specific RNAi silencing response in the target cell(s).
- RNA hairpins are thus generated under the control of tissue- or cell-specific drivers, allowing targeted knockdown of gene of interest in specific cell population(s).

RNAi can be induced experimentally by exogenous insertion of dsRNA or designed constructs which express shRNAs..

Lethality

- 5-10% of the TRiP stocks are homozygous fatal.
- fatal stocks are preserved by crossing over **balancers****
- non-fatal but unhealthy stocks are also preserved over balancers to protect the integrity of the stock

Expressing TRiP lines

- any GAL4 line can be used to activate transcription of a TRiP hairpin under UAS control
- without a GAL4 driver present you should not get expression of the hairpin and thus you should not see any knock down phenotype
- if phenotype observed in an otherwise wildtype fly, then the transgene is somewhat "leaky" (this is rare)

Enhancing knockdown with **UAS-dicer2**

- for TRiP stocks, maximum knockdown can be attained if UAS-dicer2 is carried in the GAL4 driver

Given the extent of efficiency and precision, RNAi is reportedly used in:

- systematic analysis of loss-of-function phenotypes induced by RNAi triggers
- developing therapies for the treatment of various viral infections, dominant disorders,
- in-vivo inactivation of gene products linked to disease progression and pathology in humans.

Here, we use **breathless>utrophin-GFP** as reporter. Utrophin, depicted as DMD-like (DMDL) or dystrophin-related protein (DRP), shares large structural similarities with that of the dystrophin gene, thus the name utrophin for “ubiquitous dystrophin”.

Dystrophin is a cytoskeletal protein, that forms a linkage between the cytoskeletal actin and a group of membrane proteins, as well as with a number of non-membranal proteins. And so, utrophin is an actin associated protein that links the cytoskeleton and the extracellular matrix.

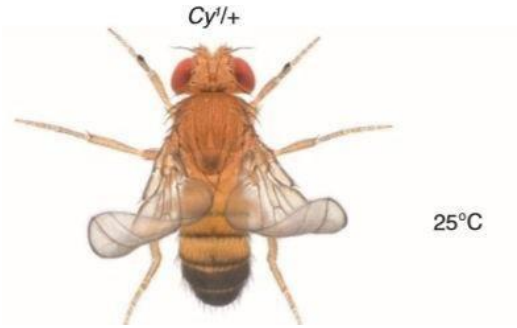
Breathless Branch(Btl), a receptor tyrosine kinase, is the mammalian FGF receptor (FGFR) homolog. Branchless (Bnl), is the homolog of mammalian fibroblast growth factors (FGF). Bnl binds to btl and predicts the direction of tracheal cell migration and consequent branch extension.

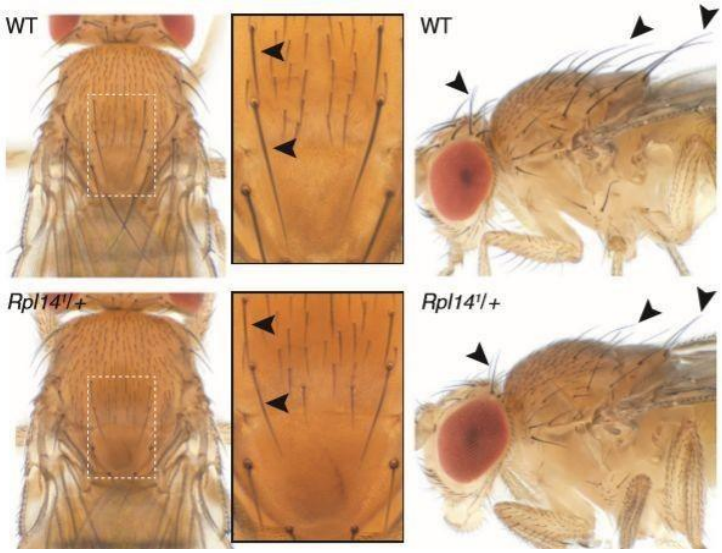
** A **balancer chromosome** is an engineered construct that ideally has three traits:

(1) one or more DNA inversions to suppress the recovery of recombination products when a recombination event occurs within the inversion during meiosis,

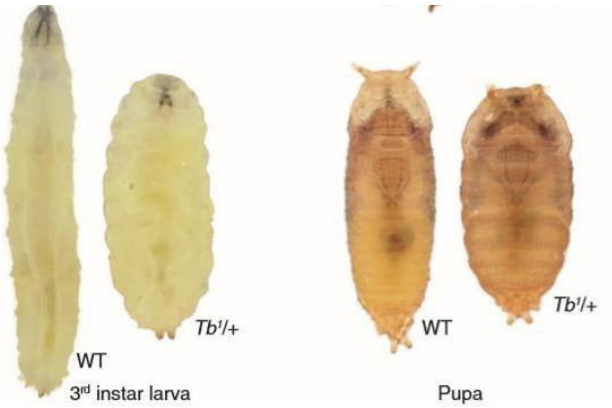
(2) a dominant phenotype that allows for visual genotyping of animals inheriting the balancer chromosome in subsequent generations, and

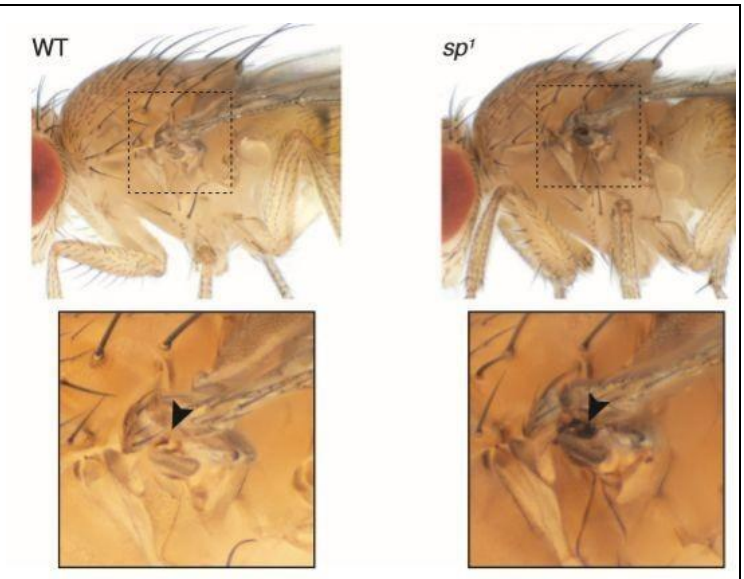
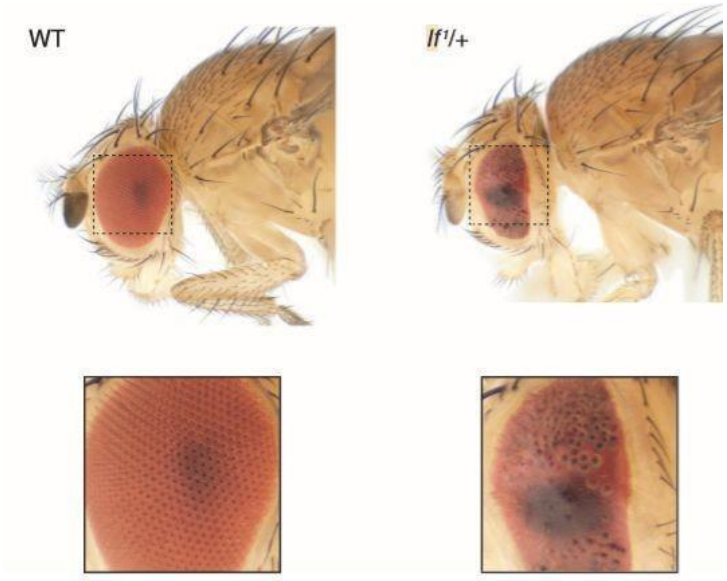
(3) either an autosomal recessive lethal mutation or an autosomal recessive mutation with an obvious phenotype to remove animals from the population that are homozygous for the balancer chromosome. [5]

balancer	**name**	**phenotype**
Curly of Oster	**CyO**	
Second Multiple 6a	**SM6a**	**curly winged phenotype**

Minute-Karmoisin-Rosy-Stubble	MKRS	
Third Multiple 6B	**Tm6b**	**tubby phenotype**

MARKERS

marker	type	phenotype
Tubby **(Tb)**	body marker	

Speck **(sp)**	body marker	
Irregular facets **(if)**	eye shape	

METHODOLOGY

I.

$$\text{♀}\ \frac{btl>utrophin{-}GFP}{CyO}\ ;\ \frac{+}{MKRS}\ \text{X}\ \text{♂}\ \frac{sp}{Sm6a}\ :\ \frac{MKRS}{TmTb}$$

Possible viable F1 progeny:

(a) $\frac{btl>utrophin{-}GFP}{Sm6a}\ ;\ \frac{+}{Tm6b}$

(b) $\frac{btl>utrophin{-}GFP}{sp}\ ;\ \frac{+}{MKRS}$

(c) $\frac{btl>utrophin{-}GFP}{CyO}\ ;\ \frac{+}{MKRS}$

(d) $\frac{btl>utrophin{-}GFP}{CyO}\ ;\ \frac{+}{Tm6b}$

(e) $\frac{sp}{CyO}\ ;\ \frac{+}{MKRS}$

(f) $\frac{sp}{CyO}\ ;\ \frac{+}{Tm6b}$

(g) $\frac{sp}{CyO}\ .\ \frac{MKRS}{Tm6b}$

screening and selection:

- Tubby and non-tubby larvae were separated.
- Fluorescent larvae were selected in each case.
- Selected larvae kept in 25°C and allowed to grow.
- Curly winged virgins are collected.

II.

$$\text{♀}\ \frac{btl>utrophin{-}GFP}{sp}\ ;\ \frac{+}{Tm6b}\ \mathbf{x}\ \text{♂}\ \frac{if}{CyO}\ ;\ \frac{UAS\ dicer}{UAS\ dicer}$$

Viable F1 progeny:

(a) $\frac{btl>utrophin{-}GFP}{sp}\ ;\ \frac{UAS\ dicer}{MKRS}$

(b) $\frac{btl>utrophin{-}GFP}{sp}\ ;\ \frac{UAS\ dicer}{Tm6b}$

(c) $\frac{btl>utrophin{-}GFP}{CyO}\ ;\ \frac{UAS\ dicer}{MKRS}$

(d) $\frac{btl>utrophin{-}GFP}{CyO}\ ;\ \frac{UAS\ dicer}{Tm6b}$

(e) $\frac{btl>utrophin{-}GFP}{if}\ ;\ \frac{UAS\ dicer}{MKRS}$

(f) $\frac{btl>utrophin{-}GFP}{if}\ ;$

$\frac{UAS-dicer}{Tm6b}$

screening and selection:

- tubby and non-tubby larvae were separated.
- Fluorescent larvae were selected in each case.
- Selected larvae kept in 25°C and allowed to grow.
- Curly winged virgins are collected.

III.

$$\text{♀}\ \frac{btl>utrophin{-}GFP}{CyO}\ ;\ \frac{UAS\ dicer}{\text{Tm6b}}\ \mathbf{x}\ \text{♂}\ \frac{if}{CyO}\ ;\ \frac{UAS\ dicer}{UAS\ dicer}$$

$$\frac{btl>utrophin{-}GFP}{CyO}\ ;\ \frac{UAS\ dicer}{UAS\ dicer}$$

- homozygous line is obtained.
- Virgins collected.
- crossed with male RNAi fly stock.

IV.

To develop an RNAi tool for in-vivo functional genomics studies in fly tracheal system.

$$\frac{btl>utrophin{-}GFP}{CyO}\ ;\ \frac{UAS\ dicer}{UAS\ dicer}\ X\ \frac{+}{+}\ ;\ \frac{UAS\ RNAi}{UAS\ RNAi}\ \mathbf{aPKC}$$

$$\frac{btl>utrophin{-}GFP}{+}\ ;\ \frac{UAS\ dicer}{UAS\ RNAi}$$

➢ cage was set-up with generated dicer line virgins and males of aPKC RNAi stock.

Controls taken for validation:

(i) $\frac{btl > utrGFP}{CyO}; \frac{+}{MKRS}$ X $\frac{+}{+}; aPKC\frac{UAS - RNAi}{UAS - RNAi}$

(ii) $\frac{btl > utrGFP}{CyO}; \frac{UAS - Dicer}{UAS - Dicer}$ self cross

- Experiment and control cages were set-up in small vials.
- Cages were kept in 29°C incubator for 48hours.
- Observations recorded.

OBSERVATIONS

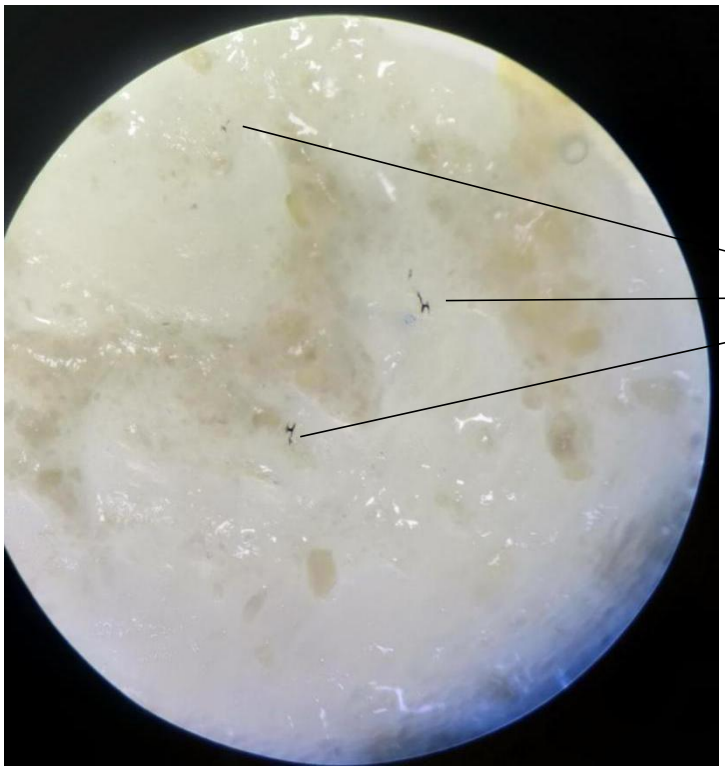


Figure 5:

Dead larvae found in RNAi cross without exogenous dicer.

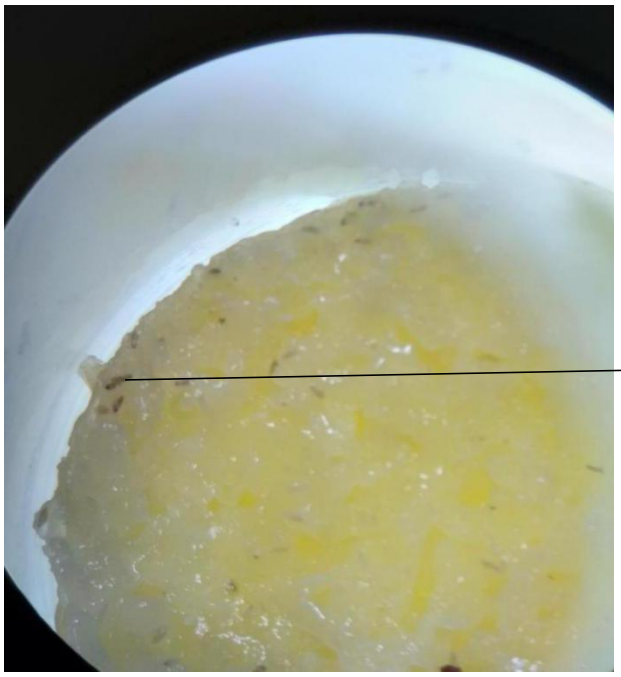


Figure 6:

Dead embryos found in the cross between RNAi line and recombinant dicer line.

RESULT

I. Dead embryos found in the cross between RNAi line and recombinant dicer line, suggest enhanced RNAi activity when provided with exogenous dicer.

II. Very few embryos hatched to larvae and grew normally

III. No dead embryo(s) found in the control RNAi cross without recombinant dicer.

IV. But, few dead larvae found in control RNAi cross without recombinant dicer, suggests RNAi activity due to endogenous dicer.

CONCLUSION

Data insufficient to draw specific conclusions.

But it appears so that there is enhanced dicer activity, when RNAi line is crossed with developed recombinant dicer line.

FUTURE DIRECTION

With the recombinant dicer line already developed and expanded, there's enough stock to design RNAi experiments:

- The same experiment can be repeated with various other RNAi lines like bazuka, shotgun, armadillo, to reproduce the results.
- Then, once the results are conclusive, suitable RNAi experiments can be designed to study in-vivo functional genomics, by knocking down a particular gene.

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To study hypoxia induced immune responses in Drosophila melanogaster.

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