Molecular Genetic Dissection of the Mechanism Underlying Cellular Plasticity in *Drosophila*

SWEETU SUSAN SUNNY

Integrated BS-MS

(Department of Biological Sciences)

IISER Mohali

A dissertation submitted for the partial fulfillment of BS-MS dual degree in Science

Indian Institute of Science Education and Research (IISER) Mohali

April 2018

Dedicated to my family

Certificate of Examination

This is to certify that the dissertation titled "**Molecular Genetic Dissection of the Mechanism Underlying Cellular Plasticity in *Drosophila***" submitted by Sweetu Susan Sunny (Reg.No.-MS13033) for the partial fulfillment of B.S.-M.S. dual degree programme of the Institute, has been examined by the thesis committee duly appointed by Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Shravan Kumar Mishra Dr. Lolitika Mandal Dr. Sudip Mandal

(Supervisor)

Dated: April 10, 2018

Declaration

The work presented in this dissertation has been carried out by me under the supervision of Dr. Sudip Mandal at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institution. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgment of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Sweetu Susan Sunny

(Candidate)

Dated: 10-04-2018

In my capacity as a supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Sudip Mandal

(Supervisor)

Acknowledgment

I am grateful to my thesis advisor Dr. Sudip Mandal for the guidance, along with sustained encouragement and motivation he has bestowed on me. His teaching, enthusiasm, and dedication to work have motivated me to pursue research.

I would like to convey my deepest gratitude to Dr. Lolitika Mandal who has been very supportive and encouraging throughout my stay in the lab. Her motivational words helped me in building up scientific temper and will surely help me in carrying myself in my interests.

I am thankful to all the lab members of Drosophila Research Laboratory namely Nidhi, Poonam, Sakshi, Ashish, Parvathy, Satish, Saikat Swati, Adyasha, Shiv, Harleen, Gunjan, Aditya, Sushmit, Prerna, Aman, Meghna and Eshita for their constant support and help.

I would like to convey my deepest gratitude to Jayati Gera who supported me throughout my stay in the lab, in all experiments and every aspect of my work.

I express most respectful regards to my thesis committee members Dr. Lolitika Mandal and Dr. Shravan Kumar Mishra for their valuable suggestions during my project.

I would like to acknowledge the former Director Prof. N. Sathyamurty, Director Prof. Debi Prasad Sarkar and Dean Research and Development Prof. Purnananda Guptasarma, for their constant support.

I sincerely acknowledge Library facility, IISER Mohali. I am thankful to Dr. P Visakhi for the help and support.

I would like to thank INSPIRE, Department of Science and Technology, Government of India for supporting me during the entire duration of my course and Indian Institute of Science Education and Research Mohali for providing me an excellent platform for learning.

I would like to express my love and care to my all friends for their love, motivation, and criticism that helped me throughout my stay at IISER Mohali.
Above all, I would like to express my most profound gratitude to my family for everlasting love and support. Thank you

LIST OF ILLUSTRATIONS

Contents

Abstract

Cellular plasticity is apparent during various developmental processes like gastrulation, organogenesis and tissue repair. Cellular plasticity includes all kind of cellular transitions like Epithelial to Mesenchymal Transition (EMT), Mesenchymal to Epithelial Transition (MET), transdifferentiation, dedifferentiation, and interconversion of different stem cell pools. Any aberrations in these biological conversions can result in disease conditions like cancer, heart failure. So it is vital to understand the mechanistic basis of these cellular transitions. For our study, we focused on understanding the molecular and genetic mechanism in Mesodermal to Ectodermal Transition using *Drosophila* as the model organism. We chose a tissue of mesodermal origin, the hematopoietic organ in *Drosophila* larvae- Lymph Gland. Overexpression of proneural gene *scute* in Posterior Signaling Center (PSC) resulted in fate change of a subset of mesodermal cells to ectodermal neurons. Furthermore, mesodermal cells undergoing cell fate change to neurons exhibit significant drop in PSC specific marker *hedgehog* and *knot*. Our results suggest that we were successful in establishing a model system to understand the mechanistic basis of mesodermal to ectodermal transition as well as epigenetic modifications and signaling pathways which prevent other cells in PSC to undergo fate change.

Chapter 1- Introduction and Objectives

1. Introduction

1.1 Cellular reprogramming

Cell differentiation has been considered as a unidirectional process for over a long period of time. Conrad Waddington depicted embryonic development as an epigenetic landscape (C.H Waddington, 1957).As shown in **Figure 1**; it represents a pluripotent stem cell as a ball rolling down a hill marked with uneven valleys and slopes. While rolling down the hill, the cell will fall into deeper inescapable valleys, representing the determined state of development and further rolls down until it reaches their most stable state, representing a differentiated cell. For several years researchers believed that a differentiated cell type can never change its fate until some reports came which challenged the belief of the field and proved that even differentiated cell can change their fate to become a different cell type.

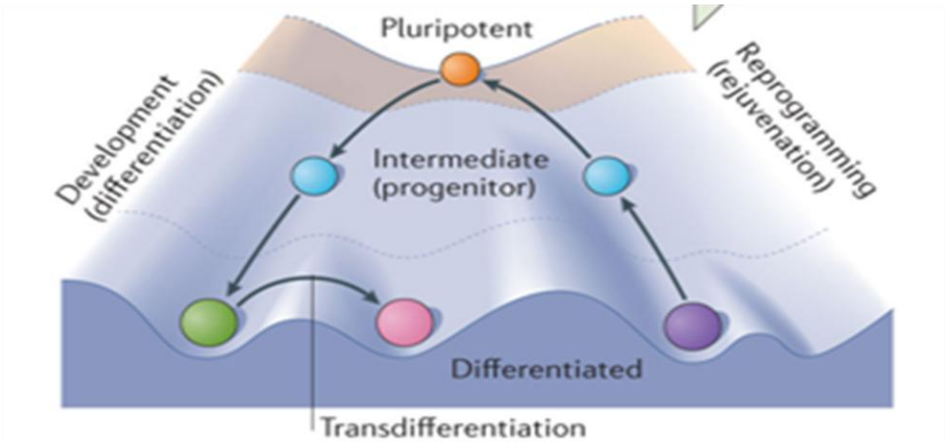


Figure 1: Waddington's landscape model

Conrad Waddington proposed a landscape model based on the idea that cell fate is irreversible. In this model, development of a cell is depicted as a ball running down the hill with uneven slopes and valleys slopes. The fate of a cell is dependent on the groove on to which it falls. In the recent past, studies have shown as that cells fate is reversible by reprogramming differentiated cells into a pluripotent state (similar to climbing up the Waddington's landscape) and by direct conversion/ transdifferentiation. (similar to crossing the ridges between valleys).(Takahashi and Yamanaka 2016)

It started with when John Gurdon proposed the concept of nuclear reprogramming by the generation of clones from somatic cells in *Xenopus laevis* (Gurdon et al., 1958). He demonstrated that a differentiated cell retains same genetic information as embryonic stem cells and has potential to develop an entire organism. Before this seminal discovery, it was

unclear that whether cells lost genetic information as they differentiate or if genes were simply turned off. Subsequently, the first evidence that mammalian nuclei could give rise to an entire organism was demonstrated by Ian Wilmut with "Dolly the sheep" (Wilmut et al., 1997). In the recent past, studies have shown that ectopic expression of some genes that are highly expressed in embryonic stem cells (OCT4, Klf4, Sox2, and c-Myc) can convert mouse fibroblasts into pluripotent stem-like cells, generally known as induced pluripotent cells (Takahashi and Yamanaka, 2006). Together, these results suggest that embryonic stem cells and fertilized eggs have some 'reprogramming factors' that can erase epigenetic memories in the somatic cell (Tada et al., 2001).

Direct conversion/ transdifferentiation

Transdifferentiation is a cellular reprogramming process in which a differentiated cell is directly converted to another differentiated cell, without complete reversion to a pluripotent state. Unlike iPSC generation where epigenetic marks are erased to attain pluripotent ground state, direct conversion aims at inducing epigenetic features of desired cell type for conversion between two unrelated cell types. So during transdifferentiation, there occurs activation and inactivation of specific genes and thus results in an irreversible switch from one cell type to another cell type.

Initial breakthrough in this field comes from some reports suggesting the role of some 'master regulator genes' in regulating commitment of cells towards some specific lineages (Takahashi and Yamanaka, 2016). Later on, reports have shown that overexpression of these master regulator genes can induce cell fate changes. Conversion of fibroblasts to myoblasts by expression of skeletal muscle factor MyoD was the first evidence for this idea (Davis et al., 1987). More recently researchers have discovered cocktail of transcription factors which can convert B and T cells to macrophages (Laiosa et al., 2006), pancreatic acinar cells to insulin-producing beta cells (Zhou et al., 2008), fibroblast into neurons (Vierbuchen et al., 2010), and fibroblast into cardiomyocytes (Ieda, 2011). Transdifferentiation process also occurs naturally during developmental process especially during regeneration after normal and physiological damage. Understanding transdifferentiation processes are important mainly because of three reasons. One is its therapeutic implications especially in case of degenerative diseases like diabetes (Zhou et al., 2008) and liver failure (Huang et al., 2011). Second is model for changes in cellular phenotype in response to certain diseases like cancer (Fang et al., 2005) and third is the identification of transcription factors involved in this process,

which also reflects the biological role of these transcription factors in understanding cellular plasticity during development.

1.2 *Drosophila* as a model system

The fruit fly, *Drosophila melanogaster* is a powerful model organism used for genetic and developmental studies for nearly over a century. This became a versatile model, after the seminal work done by T.H.Morghan and his students on chromosome theory of inheritance during the beginning of 20th century (Morgan, 1910).

Drosophila is a holometabolous insect having distinct developmental stages as embryo, larvae, pupae and adult fly. Growth and development of *Drosophila* are dependent on temperature. At 25ºC, embryos hatch out to first instar larvae within 24 hours of egg laying. *Drosophila* larval development is accompanied by molts. The period between each molt is called an instar. First instar larvae molt to develop into second instar larvae in 24 hours. After subsequent 24 hours, the second molt occurs and develops into third instar larvae. Larvae feed on provided food (foraging stage) during their development and third instar larvae continue feeding food for around 48 hours, later moves out of food to the relatively dry region (wandering stage) and eventually pupates on the wall of the vial or bottle in which they are being reared. During the pupal stage, metamorphosis takes place by replacing most of the larval structures with adult structures and last for 3-4 days. The adult flies emerge out from the pupal case and have a lifespan of about six weeks. (**Figure 2**)

Drosophila has four pairs of chromosomes (one pair of sex chromosome and three pairs of autosomes) and nearly 14000 genes (Adams et al., 2000). *Drosophila* genome was sequenced entirely by the year of 2000 (Adams et al., 2000) and comparative studies of human and fly genome have shown that about 75% of known human disease genes have a recognizable match with fruit fly genome (Reiter et al., 2001). This reveals why *Drosophila* is extensively used as a model organism for biomedical research purpose.

Drosophila system has powerful genetic tools (including Gal4-UAS, FLP-FRT, and RNAi systems) which provide profound insights into the role played by individual factors in a biological process. Now it is relatively easy to generate transgenic flies, which can inhibit or activate expression of genes of interest in a spatiotemporal manner or throughout the organism. Furthermore, a large variety of mutant *Drosophila* strains and transgenic lines are

easily available from the stock centers as well as from the laboratories that have generated them.

Drosophila researchers make use of modified chromosomes called Balancer Chromosomes for studying recessive lethal or recessive sterile mutation. Balancer has multiple overlapping inversions which prevent homologous recombination and a lethal recessive mutation which prevents the stocks from becoming homozygous for the balancer chromosome. Presence of dominant visible marker allows tracing them for generations (Greenspan R.J, 1997).

In short, having short generation time, ease of maintenance and culture, high fecundity, small and completely sequenced genome, and amenability to genetic screens makes *Drosophila* an attractive and efficient model system (Ashburner M, 1989).

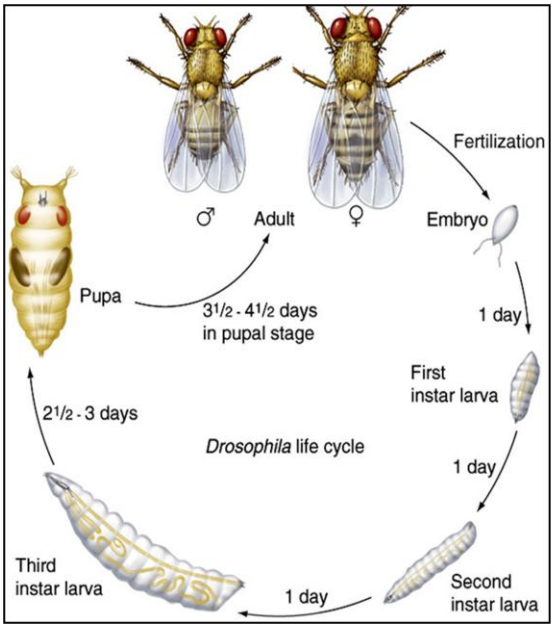


Figure 2: Life cycle of *Drosophila melanogaster*

Drosophila has four distinct developmental stages as an embryo, larvae, pupae and adult fly. Larval stages are the immature stages which are significantly different from the adult. After growing and undergoing several molts, larvae enter the pupal stage. During the pupal stage, most of the larval structures are replaced by adult structures. After 3-4 days, flies emerge out of the pupal case.

1.2.1 *Drosophila* as a model for cellular reprogramming

In a multicellular organism, master regulators maintain the identity of individual cell types and its regulation is not irreversible. Mis-expression of master regulators could induce a change in the fate of distinct cell types. This rarely happens during normal development.

Cellular reprogramming in *Drosophila*

Drosophila imaginal discs are the epithelial sac-like organs present within developing larva which forms primordia for adult fly appendages and cuticular structures. Imaginal discs are formed during embryogenesis and maintain determined state throughout larval development (Simcox. A. A, Sang J.H, 1983). During morphogenesis, in response to hormonal cues disc cells terminally differentiate to form adult fly structures (Fristrom, D. Fristom J, 1993). Many researchers have tested disc specific determination via transplantation (Gehring and Schubiger, 1975) and in vivo culture of imaginal discs. Majority of disc cells maintained their disc specific determination. However, there are rare instances where these disc cells increased their developmental potential and changed their fate (E. Hadorn, 1963). This process of cell fate change between determined cell types is called transdetermination.

Genetic analyses in flies revealed that homeotic mutations are associated with fate alteration of imaginal disc structures (Gehring and Schubiger, 1975), indicating that misexpression of homeotic genes in imaginal discs could induce transdetermination. First evidence for this came from misexpression studies of Homeobox gene *Antp*, which induced ectopic expression of legs instead of antennae (Schneuwly et al., 1987). Since then, researchers demonstrated several other examples of transdetermination in flies. For instance, ectopic eyes and wings were generated by misexpression of the genes *eyeless* (Halder et al., 1995) and *vestigial* (Kim et al., 1996) respectively. Likewise, ectopic expression of the *cut* gene in embryos resulted in the transformation of internal chordotonal organs into external sensory organs (Blochlinger et al., 1991).

1.2.2 Lymph Gland; a tissue of mesodermal origin

Drosophila hematopoiesis occurs in a specialized larval organ called lymph gland (Rugendorff et al., 1994). A matured lymph gland present in third instar larva is a multi-lobed structure that originates in the cardiogenic mesoderm of the embryo.

During *Drosophila* embryonic development, lateral mesoderm gives rise to dorsal mesoderm, which in turn gives rise to cardiogenic mesoderm and visceral mesoderm. Specification of cardiogenic mesoderm requires input from Notch (N), Wg, Dpp, and Htl signaling pathways. Cardiogenic mesoderm then differentiates into vascular cells (cardioblasts), lymph gland and excretory cells (pericardial nephrocytes). Homeobox protein Tin and GATA factor Pnr expresses ubiquitously in cardiogenic mesoderm to stage 12 of embryogenesis (Mandal et al.,

2004). Notch-mediated signaling restricts its expression to cardioblasts, and at the same stage, the zinc finger protein Odd-skipped (Odd) expresses in T1-A6 segments. Of these, thoracic clusters (T1-T3) come together to form lymph gland progenitors, and activates Zinc finger transcription factor Serpent (GATA family), whereas abdominal clusters form the pericardial cells (Mandal et al., 2004). Primordial lymph gland has two primary lobes with approximately 20 cells in each; it proliferates and increases in number of cells to about 200 prohemocytes in the second instar larval stage. At this stage, secondary lobe starts developing between pericardial cells and cells in primary lobe proliferate further to form 2000 cells. This forms matured multi-lobed lymph gland structure in third instar larvae (Jung et al., 2005) (**Figure 3**).

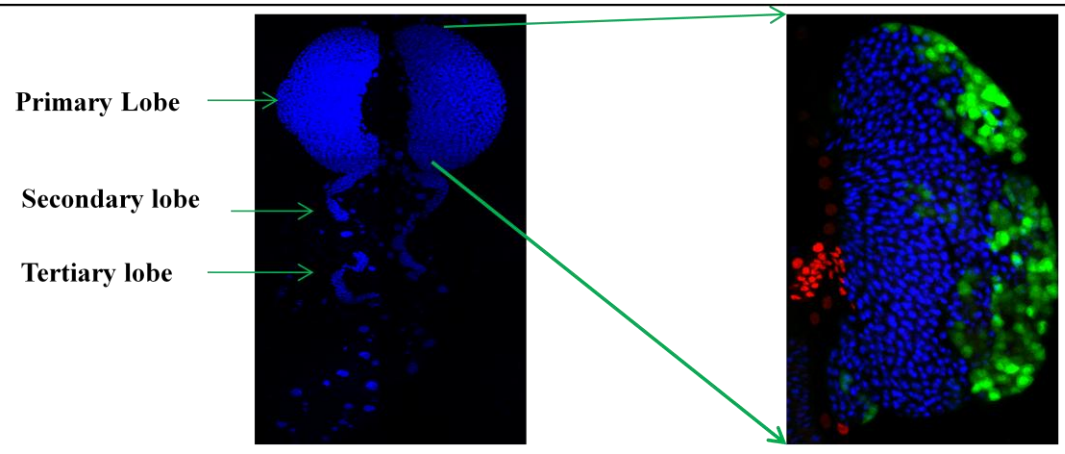


Figure 3: Lymph Gland , the hematopoietic organ in Drosophila Larvae

Figure 3a: Multi lobed lymph gland structure of third instar larvae

Figure 3a': Primary lobe of Lymph gland

Cortical Zone (CZ) - Population of differentiated hemocytes

Medullary Zone (MZ) - Population of hemocyte progenitors

Posterior Signaling Center (PSC) - Acts as a niche that maintains balance between differentiated hemocytes and progenitor cells

The primary lobe of lymph gland is much well characterized and it has three zones- outer Cortical Zone (CZ), inner Medullary Zone (MZ) (Jung et al., 2005) and the Posterior Signaling Center (PSC) (Jung et al., 2005). The outer cortical zone consists of differentiated hemocytes (Jung et al., 2005). The inner medullary zone homes for progenitors of hemocyte and posterior signaling center (PSC) consists of 45-50 cells, which acts as a niche that maintains a balance between progenitors and differentiated hemocytes (Mandal et al., 2004).

Niche cells are specified during embryogenesis by expression of Homeobox protein Antennapedia (Antp) (Mandal et al., 2007). Signaling mediated from niche through secreted Hedgehog (Mandal et al., 2007) and JAK/STAT pathway (Jung et al., 2005) are important for the maintenance of prohemocytes. Majority of the secondary and tertiary lobes of lymph gland lacks maturation markers and express markers of progenitors, suggesting that these lobes also homes for progenitors (Jung et al., 2005).

A significant degree of conservation is present between *Drosophila* hematopoiesis and vertebrate counterpart in terms of transcription factors and signaling pathways. Along with this, lymph gland is a model system which can be well documented, easily culturable and can be genetically manipulated in a cell-specific manner. This makes *Drosophila* lymph gland as an efficient model system for reprogramming studies.

1.3 Neurogenesis in *Drosophila*

During early development in *Drosophila*, embryonic ectoderm gives rise to epidermal ectoderm and neuroectoderm. Subsequently, a subset of neuroectodermal cells called neuroblasts moves inside the embryo to build up central neural primordium (Hartenstein and Campos-Ortega, 1984), whereas remaining cells form epidermoblasts (progenitors of the epidermis). The peripheral nervous system develops from the neural progenitors within the epidermis (Younossi-Hartenstein and Hartenstein, 1997). However, the molecular mechanisms involved in cell fate choice being shared in the development of the Central Nervous System (CNS) and peripheral nervous system (PNS).

The process of neurogenesis is regulated by two sets of genes - Proneural genes and neurogenic genes

Proneural proteins are bHLH transcription factors or E-proteins which form a heterodimeric complex with DNA (Cabrera and Alonso, 1991). Proneural proteins specifically bind to hexanucleotide motifs CANNTG in DNA called E-box (Murre et al., 1989). Proneural genes include genes of Achaete-Scute complex, Daughterless, Atonal and other unidentified ones.

Achaete-Scute Complex

The achaete-scute complex consists of four related genes, Achaete (ac), Scute (sc), Lethal of scute (lsc), and Asense (ase) (Gonzalez et al., 1989), (Garcia-Bellido, 1979). Achaete and Scute genes are required for generation of neuroblasts and embryonic and adult sensory organs; whereas Lethal of scute is restricted to central neural primordium (Jimenez and

Campos-Ortega, 1990). Asense is expressed in all neural progenitors, and it is a neuronal precursor gene (Jarman et al., 1993), (Dominguez and Campuzano, 1993).

1.3.1 Mechanisms of proneural activity

In *Drosophila*, neuroectoderm cells have potential to become either neuron or epidermis (Cubas et al., 1991). Initially, these cells express low levels of proneural genes (Garcia-Bellido, 1979). However, lateral inhibition mediated by Notch signaling pathway selects one cell as neuroblasts/ neural progenitor and express a high level of proneural genes, whereas other cells adopt epidermal fate, with down-regulation in proneural gene expression (Skeath and Carroll, 1992).

Notch signaling

Transmembrane receptor Notch and its ligand Delta form major components in Notch signaling pathway. Previous studies have demonstrated that activation of Notch receptor by its interaction of Delta initiates intracellular signaling cascade that inhibits proneural activity(Artavanis-Tsakonas et al., 1999). Ligand-mediated proteolytic cleavage of Notch receptor results in the release of Notch Intracellular Domain (NCID). NCID translocates to the nucleus, acts as a coactivator with Suppressor of Hairless (SuH) and activates transcription of *Enhancer of Split[E(spl)]* genes, which in turn inhibits proneural genes. (**Figure 4**)

Proneural genes, in turn, have a positive regulation on Delta and if Notch signaling inhibits proneural expression, then Delta expression is also inhibited. Stochastic changes in gene expression result in upregulation of Delta in one of the neuroectodermal cell (prospective neural progenitor). Positive feedback loops amplify this difference. Proneural genes induce transcription factors like Zinc finger protein Senseless which in turn upregulates proneural gene expression and thus upregulation of Delta. Notch signaling cannot be activated in cells with high levels of Delta because of cis inhibitory interactions with the receptor (Heitzler and Simpson, 1993). This in turn, results in specification of signal sending cells and signal receiving cells which later on differentiates to neuronal and epidermal fate respectively.

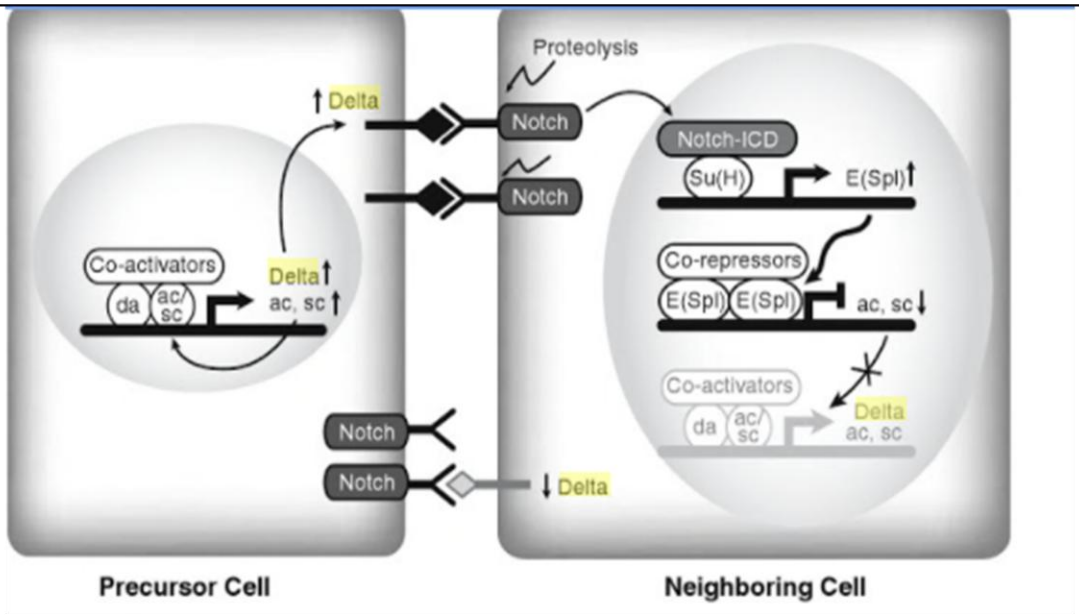


Figure 4: Lateral inhibition by Notch signaling pathway

Cells within the neuroectoderm express proneural factors achaete (ac) and scute (sc), which dimerizes with another proneural factor daughterless (da), regulates expression of Delta. Delta in turn activates Notch signaling in neighboring cell and thus inhibits proneural gene expression. This results in inhibition of neural fate in neighboring cells.

(Image copyright @ Developmental Neurobiology, Mahendra S. Rao, Marcus Jacobson)

1.4 Genetic tools used in this study:

1.4.1 Gal4 - UAS system

Gal4 is a transcription activator identified in yeast Saccharomyces cerevisiae, which binds to DNA sequence called Upstream Activator Sequence (UAS) and activates transcription of Gal4 targeted genes. Andrea Brand and Norbert Perrimon adopted this system to *Drosophila* for ectopic expression of the gene of interest in a directed manner. In *Drosophila*, Gal4-UAS system is an efficient bipartite approach in which Gal4 with the tissue-specific promoter in one transgenic line called driver line and UAS with the specific gene of interest on another fly line, responder line. When these transgenic lines are crossed, F1 progeny will contain both Gal4 and UAS elements in a single organism. As a result, Gal4 protein will go and bind to UAS and activates transcription of the target gene which downstream of UAS element (**Figure 5**).

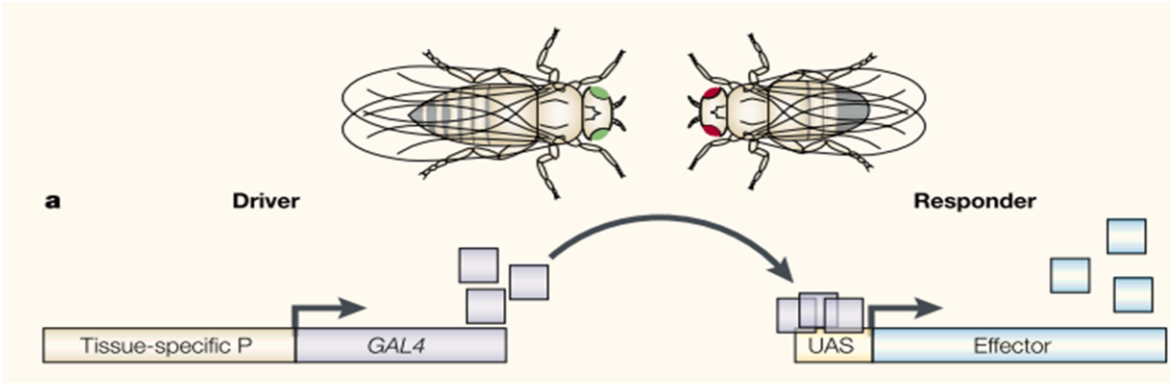


Figure 5: The Gal4 – UAS system in *Drosophila*

The Gal4- UAS system in *Drosophila* allows targeted gene expression. Driver line has Gal4 with tissue-specific promoter whereas responder line has Upstream Activator Sequence(UAS) with a specific gene of interest. When these lines are crossed, F1 progeny will have both elements and this allows expression of our gene of interest, in a tissue-specific manner.

(Copyright from http://hawaiireedlab.com/wpress/? p=477)

Using this approach, the expression pattern of a target gene can be spatiotemporally controlled using enhancer of the appropriate gene in driver line. Another advantage of this system is that it allows expression of lethal genes for a short window of time in specific tissues and excludes it effects in early developmental stages. Gal4-UAS system is temperature dependent, in which Gal4 mediated transcription of UAS, is not active at 16ºC, whereas it shows its maximum activity at 29º C and additional temporal regulation is gained by Gal80. Gal80 binds to the activation domain of Gal4 and prevents Gal4 from binding to UAS at 18ºC whereas at high-temperature Gal80 remains inactive.

2. Objectives

The primary focus of this project is to check the possibility of change in the fate of a cell of mesodermal origin, to ectodermal neurons in *Drosophila melanogaster*.

If this is true, then next aim is to check whether there is any kind of heterogeneity in the response of cells in changing fate or not?

Finally to understand the molecular and genetic mechanisms involved in this cell fate transition.

Chapter 2: Materials and Methods

1. Materials and methods

1.1 Rearing of flies and maintenance

Most of the *Drosophila* stocks used for this study were obtained from different stock centers and when required new lines were generated by crossing or recombining flies of desired genotypes. The flies were reared on food made from cornmeal, agar, yeast, and fungicides. The flies were maintained at 25ºC in standard bottles/ vials if not mentioned otherwise.

1.2 Fly stocks and genotypes

Oregon R: Wild-type laboratory stock of *Drosophila.*

Following transgenic lines were used:

ZCL 1973X: This is a GFP protein trap line on the first chromosome for an Extracellular Matrix (ECM) protein Trol. Trol/Perlecan is expressed in the hematopoietic organ, lymph gland. Procured from Vienna Drosophila Research Center.

w; kn-Gal4*:* This fly line has Gal4 insertion on the third chromosome which is under the control of DNA sequences in or near the Collier/Knot promoter. Knot /Collier (EBF homolog) is a transcription factor which is required for head patterning, the specification of muscle and neural identity during embryogenesis in *Drosophila*. In larvae, it plays a crucial role in lymph gland, under normal and stress conditions. This driver is expressed in the Posterior Signaling Center (PSC) and posterior lobes of larval lymph gland.

domeless-Gal4*:* This is a transgenic line which expresses Gal4 under the control of DNA sequences in or near *Domeless*(*Dome*) promoter. Domeless (Dome) is a transmembrane receptor for JAK-STAT signaling. In *Drosophila* larval lymph gland, Dome expresses in Medullary Zone (MZ), which homes for hemocyte progenitors. This stock is a bountiful gift from Prof. Utpal Banerjee, Department of Molecular, Cell and Developmental Biology, University of California.

w; hhF4f-GFP*:* This transgenic fly line contains 1020bp *hhF4f* enhancer DNA that drives GFP expression in PSC of larval lymph gland. This stock was a kind gift from R. Schulz, Notredame, USA

w; UAS-sc: This fly line expresses *scute* under the control of UAS on the second chromosome. Procured from Vienna Drosophila Research Center.

dome -MESOEBFP/FM7: In this fly line, blood progenitor cells in the Medullary Zone of larval lymph gland expresses Enhanced Blue Fluorescent Protein 2 (EBFP2) under the control of dome Meso enhancer. The dome Meso enhancer is a mesoderm-specific enhancer present in the first intron of *dome* gene (Hombria et al., 2005). This stock is a generous gift from Prof. Utpal Banerjee, Department of Molecular, Cell and Developmental Biology, University of California.

Transgenic lines generated in our laboratory:

dome-Gal4, UAS-GFP/FM7; tub-gal80^{ts} / tub-gal80^{ts}: In this fly line, blood cell progenitors in the Medullary Zone(MZ) of larval lymph gland expresses Green Fluorescent Protein (GFP) under the control of *dome*-Gal4. We used this to check the possibility of cell fate transition in MZ in response to *scute* overexpression.

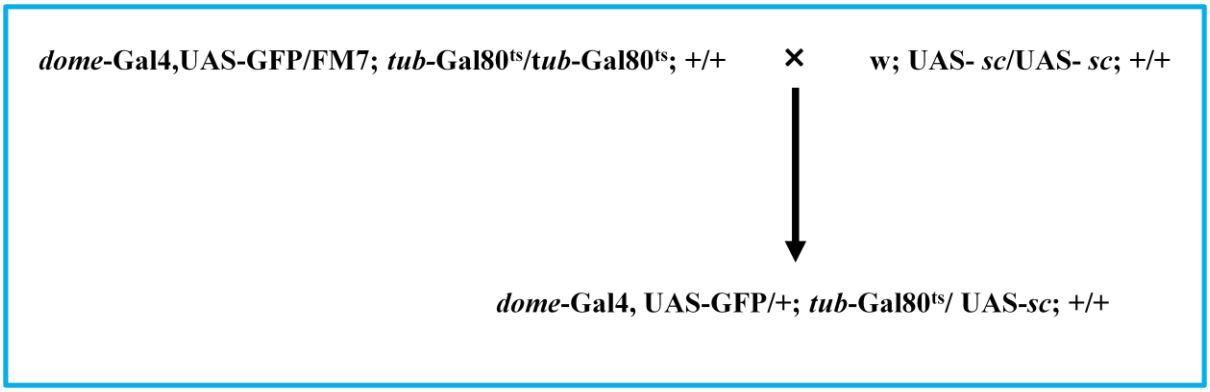
w; UAS-2XeGFP/Cyo; kn-Gal4/Tb: To visualize expression pattern of *knot* in *Drosophila* larval lymph gland, we generated a transgenic line in our laboratory by bringing GFP along with *knot* driver.

w; hhF4f-GFP/Cyo; kn-Gal4/Tb: In *Drosophila*, Hedgehog is known to be essential for ommatidial differentiation. To understand the role of *hedgehog* in cell fate transition we generated a transgenic line by bringing *hedgehog*-GFP along with *knot* driver.

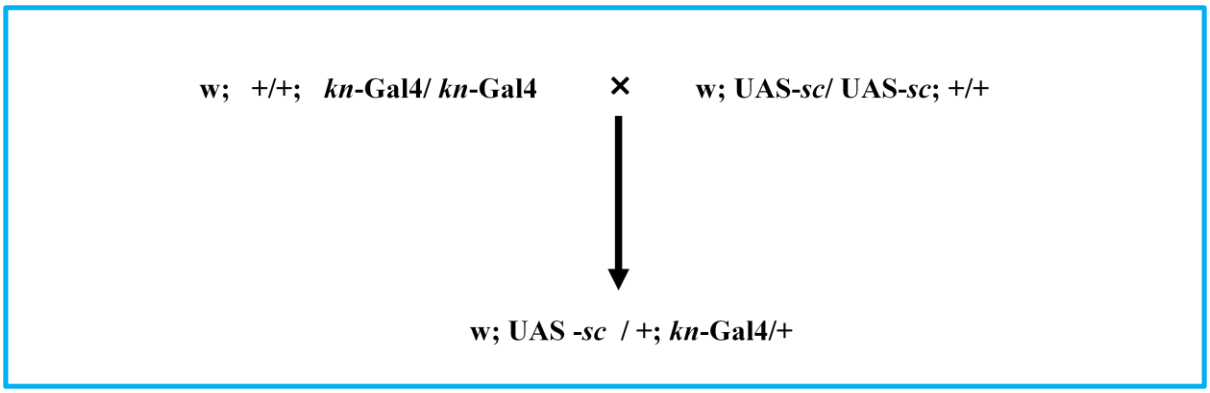
.***ZCL1973/ZCL1973; +/+; kn-Gal4/Tb***: Trol is an Extracellular Matrix (ECM) protein which forms basement membrane in animal cells. Trol forms discrete compartments within the lymph gland. To check compartment specificity of cells undergoing fate change to neurons, we generated a transgenic line, by bringing GFP protein trap line ZCL1973 with *knot* driver.

1.3. Genetic Crosses and Recombination

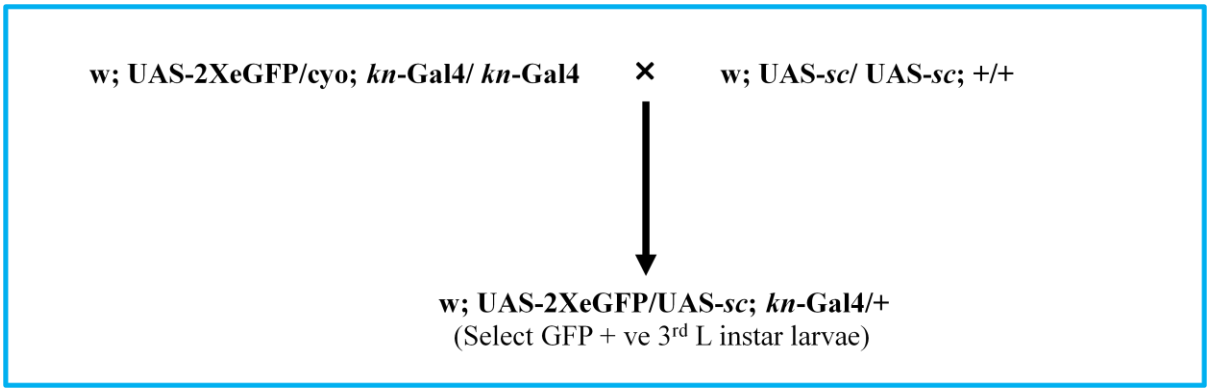
1.3a Scheme for overexpression of *scute* in Medullary Zone (MZ)



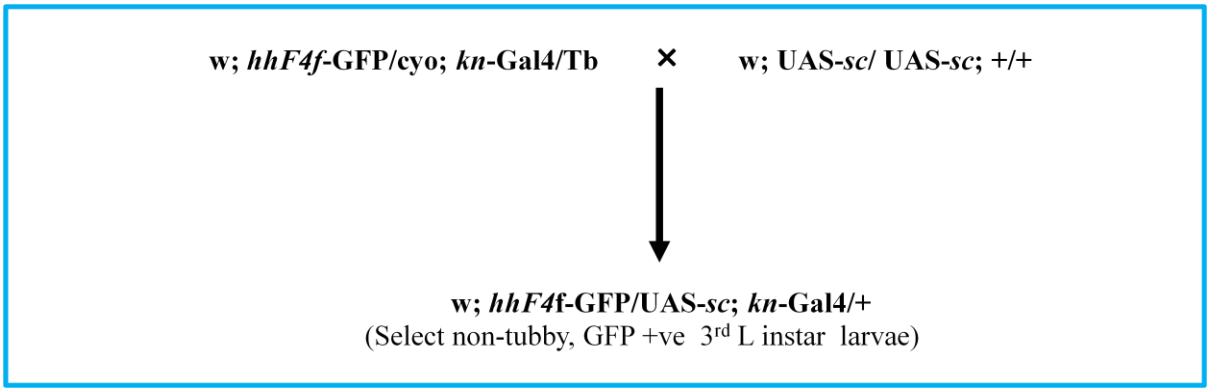
1.3b Scheme for overexpression of *scute* in the Posterior Signaling Center (PSC)



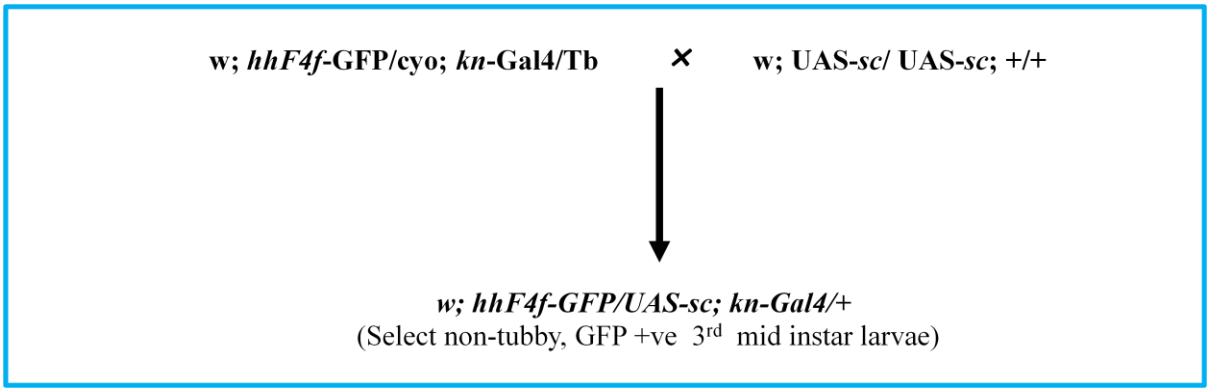
1.3c Scheme for analyzing Achaete expression in PSC in response to *scute* overexpression



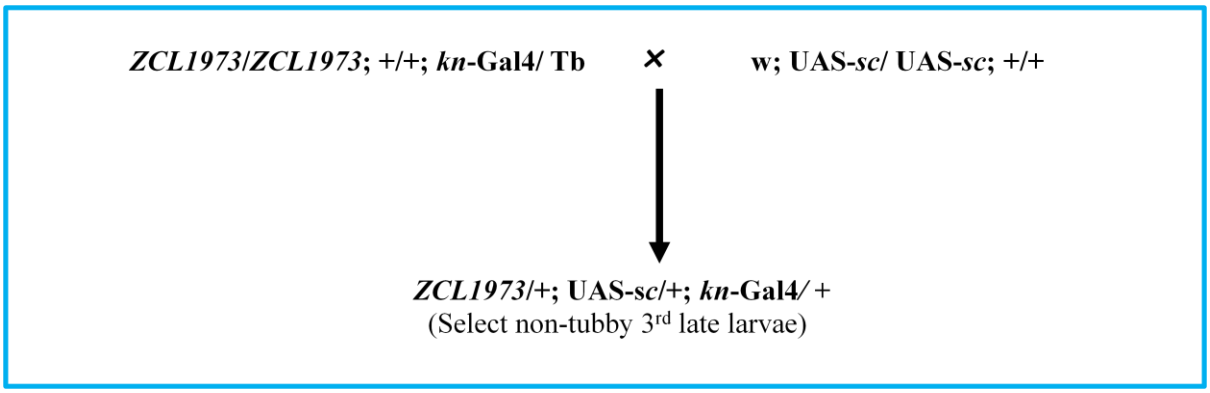
1.3d Crosses for analyzing expression of *hedgehog* in PSC during the cell-fate transition



1.3e Crosses for analyzing mesenchymal to epithelial transition in PSC during cell fate change.

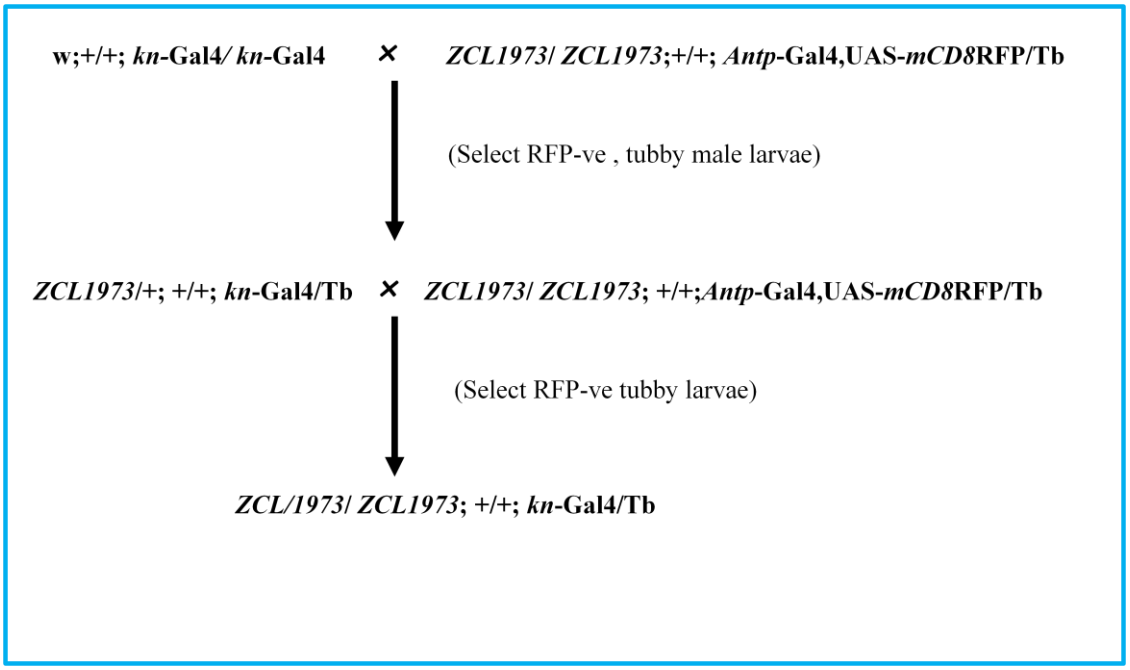


1.3f Crosses for analyzing compartment specificity of differentiating neurons in PSC



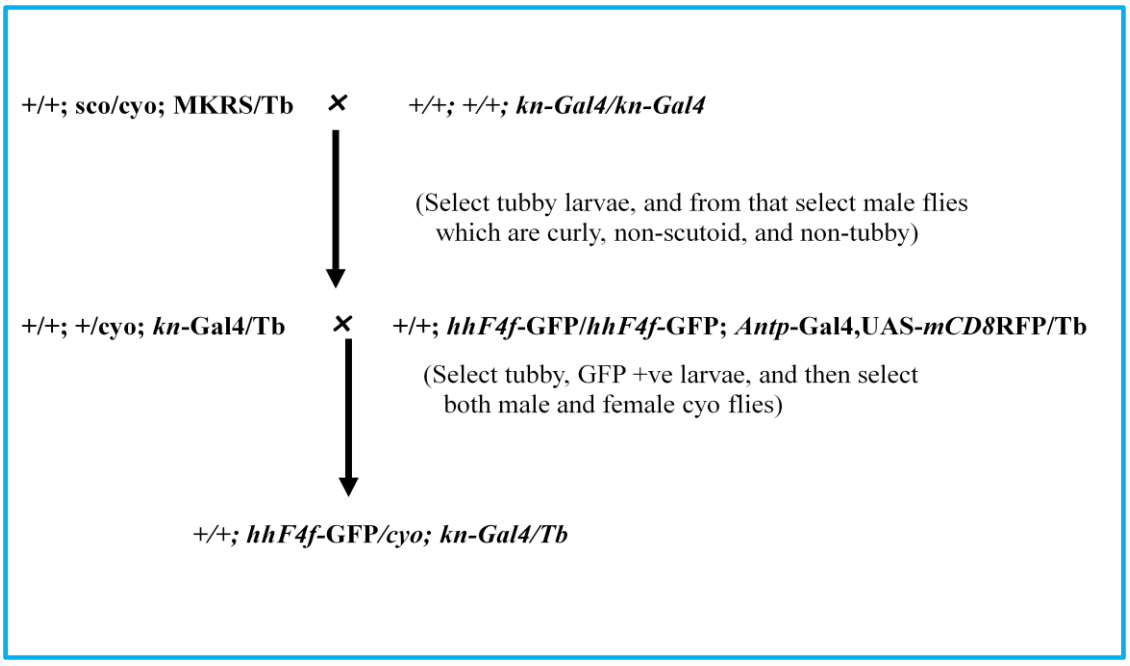
1.3g Generation of recombinant line - *ZCL1973/ZCL1973*; +/+; *kn*-Gal4/Tb

The aim of creating this line is to bring GFP trap line ZCL1973 under *knot* driver.



1.3h Generation of recombinant line- *w; hhf4F-GFP/cyo; kn-Gal4/Tb*

The aim of creating this line is to bring *hhF4f*-GFP under *knot* driver



Note: For all of the crosses, egg-laying bottles were kept at 29ºC and flies were kept at 25ºC

1.4 Immunohistochemistry

Late third instar larvae were dissected in 1X PBS followed by fixation in 5% paraformaldehyde for 40 minutes followed by three washes of 15 minutes each with 0.3%PBT at room temperature. Tissues were incubated in blocking solution, 10% NGS (Normal Goat Serum) for 1 hour and kept on a shaker. Once blocking were done, the sample was incubated with primary antibody for 16-18hours at 4ºC (All primary antibodies, source, and its dilution are mentioned below).

After incubation, tissues were washed three times for 15 minutes each using 0.3%PBT at room temperature. Before secondary antibody tissues were incubated in 10% NGS for 30 minutes (All secondary antibody, source, and dilution used are mentioned below). Following this tissues were incubated in secondary antibody for 16-18 hours at 4ºC. Tissues were given three washes for 15minutes each with 0.3% PBT followed by two washes with 1XPBS of 10 minutes each. Then tissues were incubated with DAPI for 1 hour. After DAPI treatment, samples were quickly washed in 1X PBS for three times of 10 minutes each and mounted in vectashield.

1.3a Primary antibodies used

For immunostaining the following primary antibodies were used:

Antibody	Developed in	Source	Antibody number	Dilution used
Anti-ELAV	Rat	DSHB, Lowa	7E8A10	1:100
Anti-Futsch	Mouse	DSHB, Lowa	22C10	1:100
Anti-Antp	Mouse	DSHB, Lowa	8C11	1:10
Anti-achaete	Mouse	DSHB, Lowa	-	1:10
Anti-DEcadherin	Rat	DSHB, Lowa	DCAD2	1:25

1.3b Secondary antibodies used

Following are the secondary antibodies used for this study:

Antibody	Source	Details
Cy^{TM3}-Conjugated AffiniPure donkey Anti-Rat IgG(H+L)	Jacksons Immuno Search laboratories the USA Code-711-165-153	Conjugated with cyanine Cy^{TM3} dye (absorption maxima/ emission maxima is 550 nm/ 570 nm) working dilution:1:500 Detect primary antibodies raised in the rat.
Cy^{TM3}-Conjugated AffiniPure Goat Anti-Mouse IgG(H+L)	Jacksons Immuno Search laboratories the USA Code-711-165-152	Conjugated with cyanine Cy^{TM3} dye (absorption maxima/ emission maxima is 550 nm/ 570 nm) working dilution: 1:500 Detect primary antibodies raised in mouse.
Fluorescein (FITC) AffiniPure Goat Anti-Mouse IgG(H+L)	Jacksons Immuno Search laboratories the USA Code -115-095-003	Conjugated with FITC (absorption maxima/emission maxima is 492/520nm) Working dilution: 1:500 Detect primary antibodies raised in mouse

1.3c Stains used

DAPI

DAPI (4',6-diamidino-2-phenylindole dihydrochloride). This is a blue fluorescent dye that binds to A-T rich region in double-stranded DNA. It is used to stain nuclei in live as well as fixed tissues. Its absorption maxima/ emission maxima are 351nm/461nm. The working dilution is 1µg/ml. Tissues after secondary antibody treatment were washed thrice for 5 minutes each with 1X PBS. Then, tissues were incubated in DAPI solution (1µg/ml) in 1X

PBS for 45 minutes at room temperature. After incubation, tissues were washed with 1X PBS for three times of 10 minutes each.

TO-PRO-3

TO-PRO-3 is a far-red fluorescent dye that has a strong affinity for double-stranded DNA. It is impermeant to live cells and can stain only nuclei for fixed/dead tissues. So generally this is used as an indicator of dead cells within a population. This carboxycyanine-based dye has excitation and emission maxima at 642 and 661nm respectively. Tissues after postsecondary washes with 0.3%PBT, tissues were washed in IXPBS for 5 minutes. Then tissues were incubated in TO-PRO-3(1:1000 in 1XPBS) for 45 minutes. After incubation tissues were washed with 1X PBS for three times of 10 minute each.

Chapter 3-Results and Discussions

1. Results

1.1 Overexpression of proneural gene *scute* in Medullary Zone (MZ) of primary lobe doesn't result in any cell fate change

To check the possibility of change in the fate of cell of mesodermal origin to ectodermal neurons, we chose the hematopoietic organ in *Drosophila* larvae - Lymph gland. We overexpressed proneural gene *scute* in Medullary Zone (MZ) of *Drosophila* larval lymph gland using tissue-specific driver *domeless.* Expression of Domeless is found in MZ of the primary lobe and in the secondary lobe of 3rd instar larval lymph gland (**Figure 6A)**. We wanted to see whether these progenitor cells undergo cell fate change to neurons, in response to *scute* overexpression. For that, we looked for ELAV (Embryonic Lethal Abnormal Vision) expression, which is a terminal differentiation marker of neurons in *Drosophila.* Overexpression of scute in progenitor cells of primary lobe of lymph gland doesn't result in any ectopic expression of ELAV in hemocyte progenitors (**Figure 6B-6C'**).

1.2 Overexpression of proneural gene *scute* in Posterior Signaling Center (PSC) results in cell fate change to neurons

We focused on next set of progenitors like cells present in secondary and tertiary lobes of lymph gland. We overexpressed *scute* using *knot (kn)*-Gal4 which express in anterior compartments of secondary and tertiary lobes and also in Posterior Signaling Center (PSC) of the primary lobe (**Figure 7A**). Surprisingly, differentiated cells of PSC have undergone the transition to neurons (**Figure 7B-7C'**) whereas; there is no ectopic expression of ELAV in secondary and tertiary lobes of lymph gland (**Figure 7D-7D'**). We found that frequency of this cell fate transition is 20% (**Figure 7E**). Importantly, there is a drastic decrease in the expression of Knot in cells undergoing cell fate change (**Figure 7F-7G''**). This suggests that cells that are undergoing cell fate change have lost one of the markers of PSC.

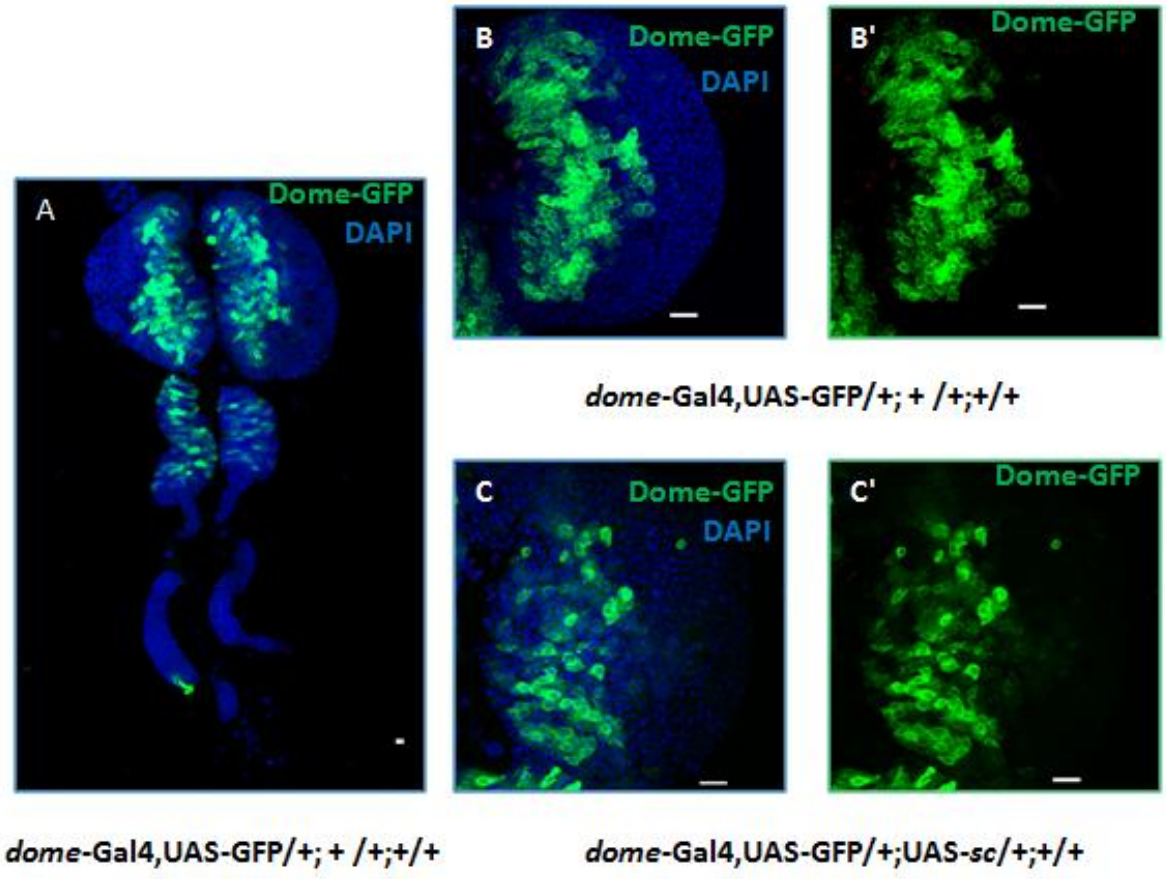


Figure 6: *scute* overexpression does not result in any cell fate change in Medullary Zone (MZ)

(A) Expression pattern of Domeless in third instar lymph gland (96hr post-hatching) visualized by GFP driven by *dome-Gal4*. Domeless expresses in Medullary Zone(MZ) and secondary lobes of lymph gland**.(B-B')** Confocal sections showing the expression of Domeless (GFP), anti-ELAV (Red) and DAPI (Blue) in the primary lobe of a control third instar lymph gland (*dome*-Gal4, UAS-GFP). ELAV marks none of the cells in the Medullary Zone. **(C-C')** The same observation is found when *scute* is overexpressed in MZ driven by *dome*-Gal4.This suggests that no ectopic neurons are formed in response to *scute* overexpression in progenitor cells. Scale bar -20µm

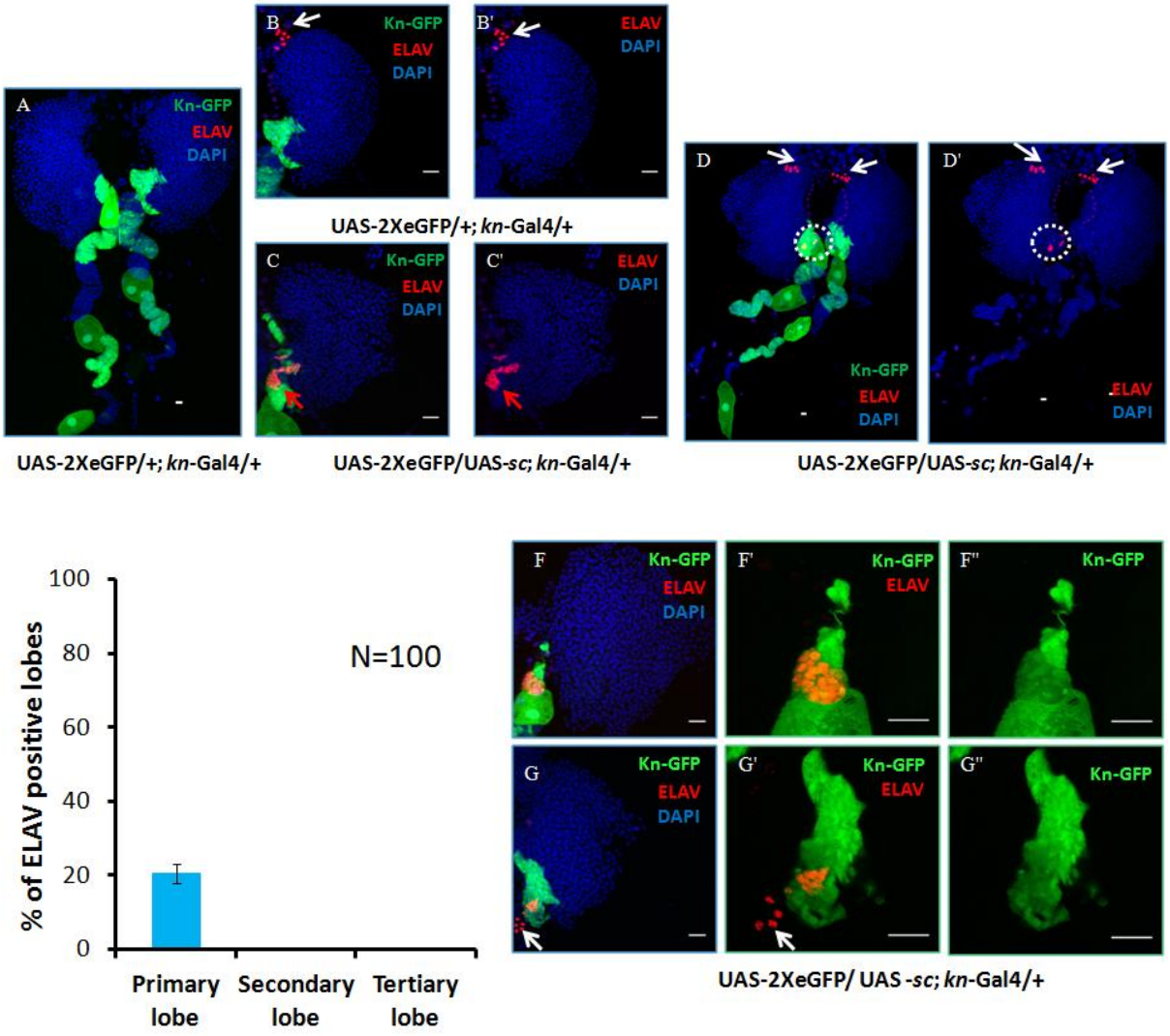


Figure 7: *scute* overexpression in Posterior Signaling Center (PSC) leads to cell fate change to neurons

(A)-Expression pattern of Knot (kn) in third instar Drosophila larval lymph gland (96 hr post-hatching) visualized by GFP which is driven by *kn*-Gal4. Knot expresses in PSC and the anterior part of secondary and tertiary lobes**.(B-B')**Confocal sections showing the expression of *kn*-Gal4(GFP), anti-ELAV(Red) and DAPI (Blue) in the primary lobe of a control third instar lymph gland (*kn*-Gal4; UAS-2XeGFP).**(C-C')**On overexpression of *scute* using *kn*-Gal4, ELAV expressing cells were found in PSC.**(D-D')**Only cells in the PSC are undergoing cell fate change, no cells in secondary and tertiary lobes are undergoing fate change to neurons.**(E)**Graph showing the frequency of this cell fate change. SD= 2.7**(F-G'')** Confocal sections showing a drastic decrease in the expression of *knot* in cells undergoing fate change. **(F'-F'')** are magnified images of PSC shown in **F**. Similarly **(G'-G')** are magnified images of PSC shown in **G** (White arrow indicates cells of ring gland **(B-B', D-D'),** Red arrow and a white circle indicates ELAV expressing cells **(D-D').** Scale Bar-20 µm

1.3 Only a subset of cells in Posterior Signaling Center (PSC) undergo cell fate change and expresses neuronal marker.

Antennapedia (Antp) is a bonafide marker for Posterior Signaling Center (PSC). We first confirmed that all *knot* expressing cells in primary lymph gland are expressing Antp (**Figure 8A-8A'**). Next, we checked whether all cells in the PSC are undergoing cell fate change in response to *scute* overexpression. Using terminal differentiation marker of neuron ELAV, we found that only a subset of cells in PSC are undergoing cell fate change (**Figure 8B-8C'**). There is variation in the number of cells in PSC which are undergoing fate transition to neurons. It can vary from 8 to 16 cells (**Figure 8B-8C'**).

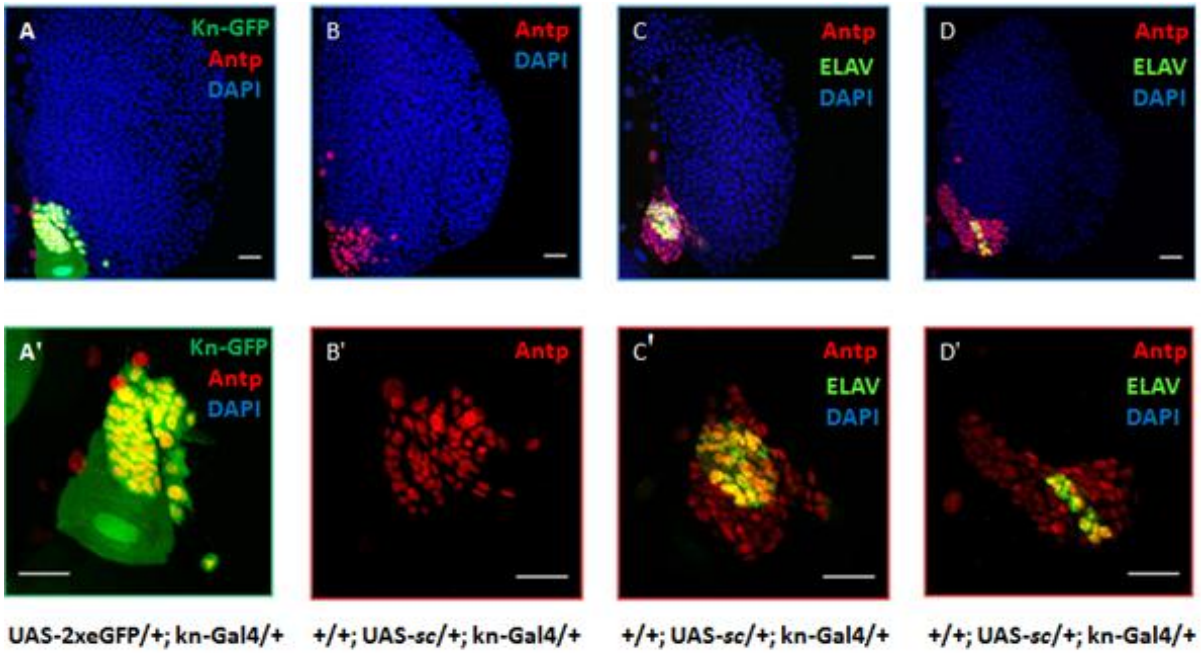


Figure 8: Only a subset of cells in PSC undergo cell fate change

(A-A') All Antp (Red) expressing cells in PSC are expressing *knot*-GFP in third instar larval lymph gland (96hr post-hatching). **A'** is the magnified image of PSC shown in **A**. **(B-B')** ELAV (Green) is not expressed in PSC of control third instar larval lymph gland. **B'** is the magnified image of PSC shown in **B. (C-D')** PSC specific scute overexpression resulted in ectopic ELAV expression (Green), but only in a subset of Antp (Red) cells. **C' and D'** is the magnified image of PSC shown in **C and D** respectively. The number of cells expressing ELAV varies from sample to sample **(C'-D').** Scale bar-20µm

1.4 Differentiated neurons undergo proper developmental morphogenesis

Axons are known to help neurons in connecting to target sites and to form synapses. Therefore, proper morphogenesis during neuronal development includes axons projecting out of neurons. In order to check whether differentiated neurons have undergone proper morphogenesis, we used a monoclonal antibody 22C10 which recognizes Microtubule-Associated Protein Futsch in *Drosophila* (Hummel et al., 2000). The 22C10 antibody is widely used for visualizing neuronal morphology and axonal projections. Using this, we have found that differentiated neurons are projecting out axons (**Figure 9**). However, the axonal projections of these neurons are very short. They do not fuse to form any neoronal connection with the dorasl or ventral lobe of the brain.

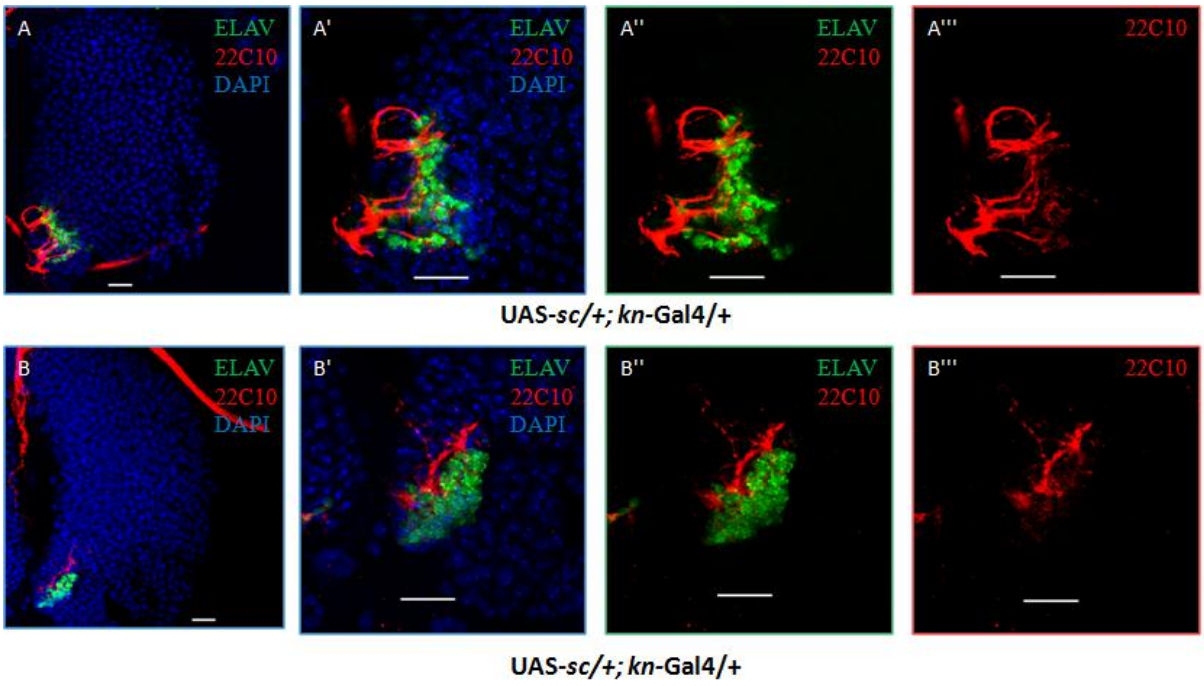


Figure 9: Differentiated neurons undergo proper developmental morphogenesis

(A-B''') Confocal sections showing 22C10 (Red), ELAV (Green) and DAPI (Blue) in the primary lobe of late third instar larval lymph gland in response to *scute* overexpression. Differentiated neurons in PSC are projecting out axons, which is marked by 22C10 antibody. **(A'-A''')** indicates magnified images of ELAV positive cells in PSC shown in **A**. Similarly **(B-B''')** indicates magnified images of ELAV positive cells in PSC shown in **B**. Scale bar-20µm

1.5 Downregulation of *hedgehog* expression in cells undergoing fate change to neurons

Hedgehog (Hh) is a morphogen secreted by cells and Hedgehog signaling network is important during embryonic development (Varjosalo et al., 2006).Hh signaling specifies neural fate in developing neural tube in vertebrates (Dessaud et al., 2008)and in *Drosophila*, it is essential for differentiation of photoreceptors in the compound eye (Roignant and Treisman, 2009) In *Drosophila* larval lymph gland, PSC secretes Hedgehog (Hh) and it allows maintenance of progenitors in the Medullary Zone (MZ). Therefore in this study, we wanted to assess the status of *hh* expression during cell fate transition of differentiated niche cells to neurons. For this purpose we used enhancer trap line *hhF4f*-GFP, to visualize the expression of Hh transcripts. As shown in (**Figure 10A-10A'''')**, we found the expression of Hh in PSC of control third instar larval lymph gland. We made a recombinant line by recombining *hhF4f*-GFP with *kn*-Gal4. We found that level of Hh got diminished in a subset of cells in PSC in response to overexpression of scute **(Figure 10B-10B'''')**. Using neuron-specific marker ELAV, we found cells that were undergoing fate change actually had a low or diminished level of *hh* expression **(Figure 10D-10D'''')**. This suggests that cells that are undergoing cell fate change are not exhibiting PSC specific marker Hh.

1.6 Differentiated neurons in Posterior Signaling Center (PSC) do not belong to one particular compartment

Extracellular matrix is an assemblage of proteins secreted by cells and these proteins provide structural and biochemical support to the neighboring cells. ECM forms a basal membrane in many of the cells and in case of epithelial cells, it lines the basal surface whereas for mesenchymal cells it surrounds the cells (Durbeej, 2010). Many of the basement membranes are composed of collagen, Laminin, Nippon, and Heparin Sulfate Proteoglycan (Durbeej, 2010). Perlecan is one such heparin sulfate proteoglycan and Trol is its homolog in Drosophila(Laurila and Leivo, 1993). In lymph gland, Trol expression is found in basement membranes that surround the surface as well as form discrete chambers within lymph gland interior(Grigorian et al., 2013).For this study, we assessed that whether cells undergoing fate change demonstrate any compartment specificity. In order to analyze compartment specificity, we utilized protein trap line ZCL1973. We made a transgenic line by bringing *ZCL1973X* along with *kno*t(kn)-Gal4 driver. We found discrete chambers within PSC which are marked by antennapedia expression **(Figure 11A-11A'''')**. Cells that are undergoing fate

change are not belonging to a particular chamber, it belongs to one or two chambers within the PSC **(Figure 11B-11C''')**.

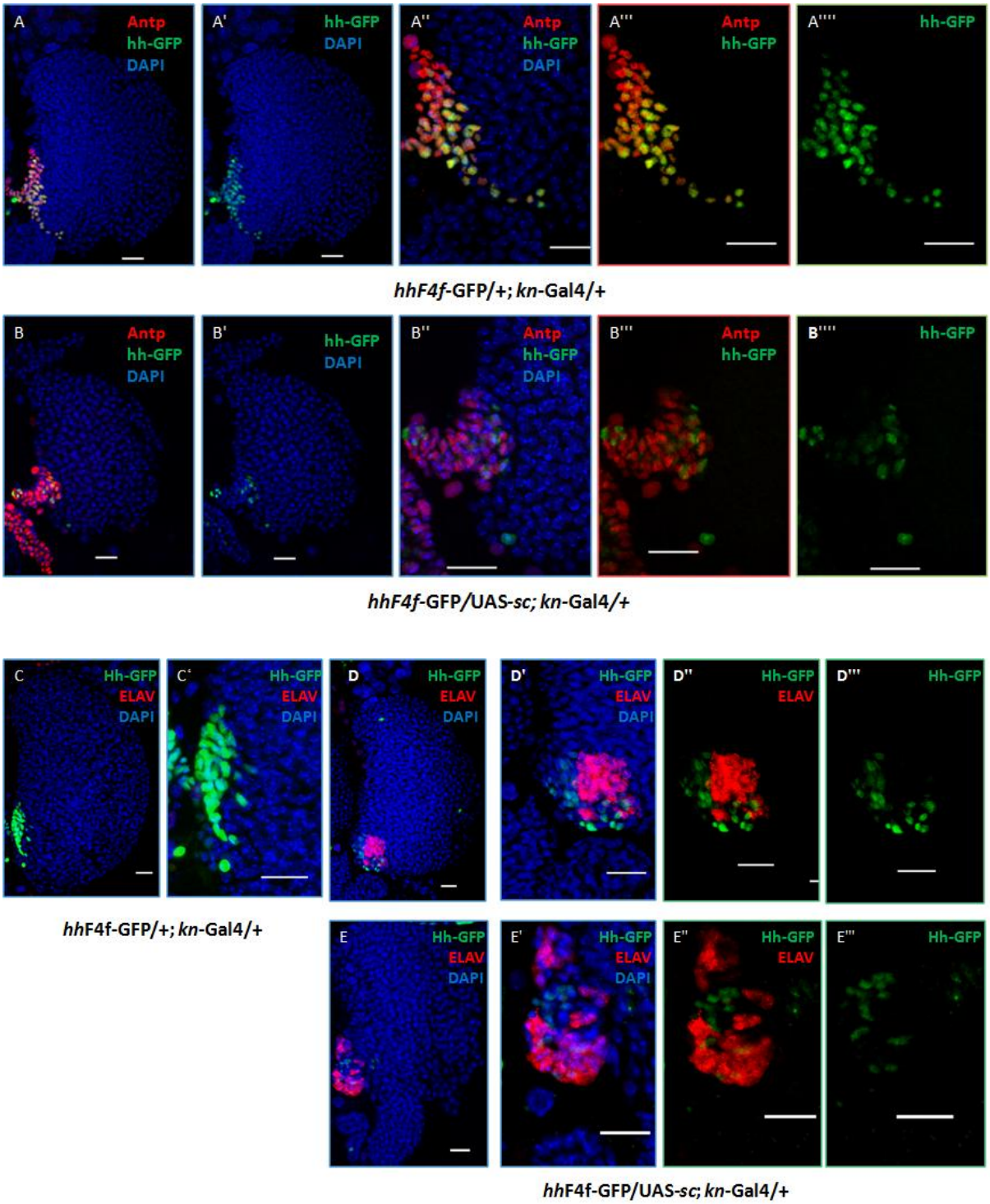


Figure 10: Downregulation of *hedgehog* expression (*hh*) in cells undergoing fate change to neurons

(A-A'''') *hedgehog* expression in control late 3rd instar larval lymph gland PSC marked by Antp (Red). **(A''-A'''')** indicates magnified images of PSC shown in **A and A.' (B-B''')** PSC

specific overexpression of *scute* resulted in decreased *hh* expression (GFP). **(B''-B'''')** indicates magnified image of PSC shown in **B and B'**. **(C-C')** ELAV (Red) and *hh* (GFP) expression in 3rd instar control lymph gland. **C'** is the magnified image of PSC shown in **C**. **(D-E'''')** There is a drastic decrease in the expression of *hh* transcripts in cells expressing ELAV. **(D'-D''')** indicates magnified image of PSC shown in **D.** Similarly **(E'-E''')** indicates magnified image of PSC shown in **E**. **D** and **E** indicates two different samples in which *scute* is overexpressed. Scale bar-20µm

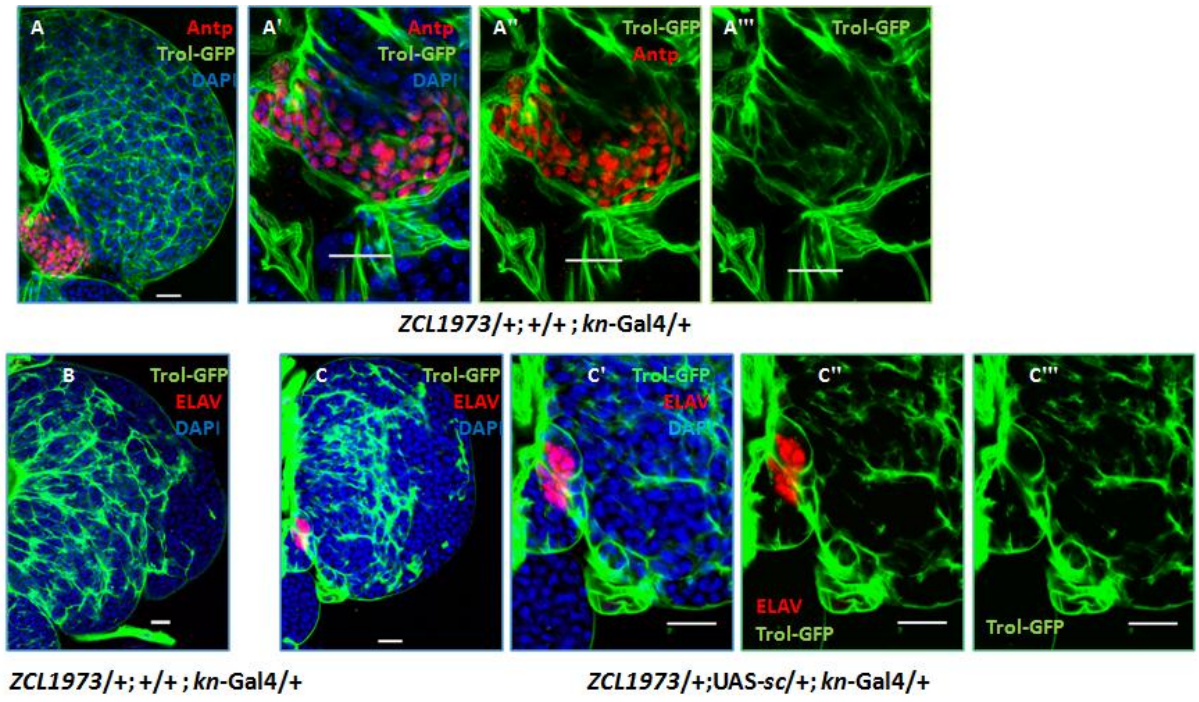


Figure 11: Differentiated neurons in Posterior Signaling Center(PSC) does not belong to one particular compartment

(A-A''') Confocal sections showing expression pattern of Trol in late 3rd instar control larval lymph gland. (**A'-A''')** indicates magnified images of PSC shown in **A. (B)** Confocal section showing expression of Trol in control 3rd instar larval lymph gland and shows that in control lymph gland there is no expression of ELAV**. (C-C''')** Overexpression of *scute* resulted in ectopic ELAV expression, and these ELAV positive cells fall in one or two chambers formed by Trol.**(C'-C''')** indicates magnified images of ELAV positive cells present in PSC of **A**. Scale bar- 20µm

1.7 Standardization of Achaete antibody

Achaete-Scute complex in *Drosophila melanogaster* consists of four related genes, Achaete (ac) , scute (sc) , lethal of scute (lse) and asense (ase). Achaete and Scute are important for the generation of neuroblasts and adult sensory organs (Garcia-Bellido and de Celis, 2009).In neuroblast cells, achaete and scute complex dimerizes with another proneural factor daughterless and thus regulates Notch signaling pathway. This, in turn, results in inhibition of neural fate in neighboring cells. For our study, we wanted to know whether all cells in the PSC expresses Achaete or only a subset of cells are expressing Achaete in response to *scute* overexpression. As a preliminary step, we standardized Achaete antibody (DSHB, Lowa) by checking its expression pattern in the Wing disc of late third instar larvae (Garcia-Bellido and de Celis, 2009) (**Figure 12A-12A'**). To ascertain the expression of Achaete in response to *scute* overexpression, we have to do immunohistological analysis using Achaete antibody in mid-third larval lymph gland.

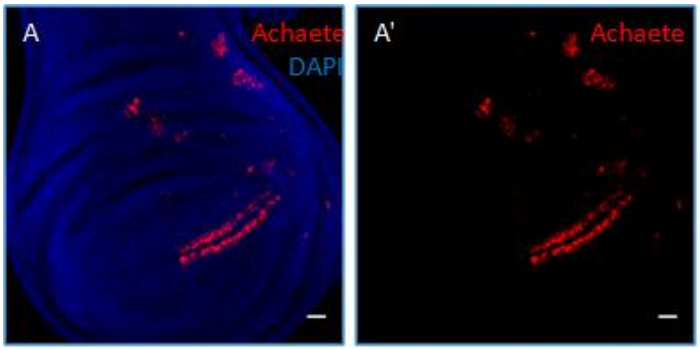


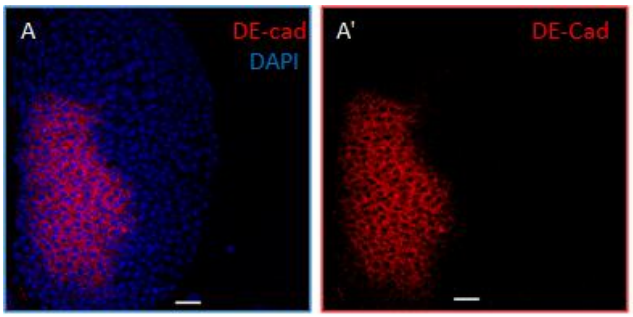
Figure 12: Expression of Achaete in wing disc

(A-A') This shows expression pattern of Achaete in wing disc of late third instar larvae. Scale bar-20µm

1.8 Standardization of DE-Cadherin antibody

E-cadherins are calcium-dependent transmembrane proteins which form principal components of adherens junction (Taneyhill, 2008),(van Roy and Berx, 2008). E-cadherin(E-cad) is involved in inducing Mesenchymal to Epithelial Transition(MET) (Auersperg et al., 1999), and it is upregulated in cells undergoing MET (Li et al., 2010). So it is widely used a marker for MET. In our study, we have shown that in response to ectopic *scute*

overexpression, some cells within the PSC of the larval lymph gland are undergoing fate change to neurons. We were therefore intrigued to know whether all cells in PSC expresses E-cad in the same level or only a subset of cells express E-cad in response to scute overexpression. As a preliminary step, we first standardized DE-Cadherin (DE-cad) antibody in third instar larval lymph gland **(Figure 13A-13A').**We found expression of DE-cad in MZ prohemocytes as reported (Jung et al., 2005).



+/+;+/+;+/+

Figure13: Expression of DE-cadherin in third instar larval lymph gland

(A-A') Confocal sections showing expression of DE-cadherin in 3rd instar larval lymph gland. Scale bar -20µm

2. Discussion

Scientists have long been fascinated by the possibility that cells can change their fate. This flexibility of cells to undergo fate change is termed as cellular plasticity. Cellular plasticity is fundamental to development and is apparent in gastrulation, organogenesis and tissue repair. Cellular plasticity includes all kind of cellular transitions like Epithelial to Mesenchymal Transition (EMT), Mesenchymal to Epithelial Transition (MET), transdifferentiation, dedifferentiation, and interconversion of different stem cell pools.

Because of high implications in regenerative medicine, cell fate change is been extensively studied using in-vitro as well as in-vivo models. *Drosophila* is widely used for understanding mechanistic basis of transdetermination (conversion of a determined cell to another determined cell) process. However, most of these studies were on conversions of one cell type to another that originate from the same dermal layers (K D McClure et al, 2007). Till now, there is no evidence showing the conversion of a cell of mesodermal origin to ectodermal origin in *Drosophila*. For this study we chose lymph gland as a tissue of mesodermal origin. The presence of three distinct zones which consists of three different populations (niche cells, hemocyte progenitors, and hemocytes) and availability of a specific driver for each zone made lymph gland an attractive model for our study.

In our study we showed that overexpression of proneural gene *scute* resulted in cell fate transition of mesodermal cells (within the PSC) to ectodermal neurons. Only a subset of cells in PSC had undergone cell fate change in spite of the fact that *scute* is overexpressed in all cells of PSC. There is a drastic decrease in the levels of PSC specific markers Hh and Knot. In addition, we found that differentiated neurons in PSC had undergone proper morphogenesis. Here we established a model which allows us to understand the mechanistic basis of mesodermal to ectodermal transition.

Understanding mesodermal to ectodermal transition is important because these transition pathways are exploited during disease conditions. Mesenchymal cells are loosely organized cells in a three dimensional extracellular matrix with reduced cell adhesion, where as epithelial cells have close contact with neighboring cells and maintain an apicobasal axis polarity. Mesenchymal to Epithelial Transition (MET) and its reverse process Epithelial to Mesenchymal Transition (EMT) are biological processes which are fundamental to embryonic development. Aberrations in these developmental pathways are highly deleterious

in adults. For example, EMT play a crucial role in the development of metastatic cancer (Thiery, 2002). Furthermore, morphological similarities between primary tumor and secondary tumor suggest that tumor cells reactivate their epithelial characteristics and this transition can be likely through MET (Chaffer et al., 2006). Because of its role in cardiogenesis, accelerating or delaying of MET can results in structural and functional heart defects (Jackson et al., 2017).So it is highly important to unravel mechanistic basis of these transitions.

Here we reported that only a subet of cells in PSC had undergone the cell fate change. This allows us to understand the factors (Epigenetic modifications, Signaling pathways) which prevent these cells from undergoing cell fate transition.

Studies in *Drosophila* have shown that Hh is involved in inititaion and propagation of retinal differentiation (Dominguez and Hafen, 1997). Based on this idea we hypothesized that there can be a connection between neuronal differentiation and Hh. But , we found that there is down regulation in *hh* level in case of cells undergoing fate change to neurons. Sonic hedgehog has been reported to induce EMT in tumors (Yoo et al., 2011). So we speculate that this reduction in *hh* expression can be due MET in cells undergoing fate change. Of transition markers like E-Cadherin. We have standarized DE-cad antibody in lymph gland of *Drosophila* larvae as a preliminary step to this.

Extracellular Matrix Protein Trol, forms chambers within the lymph gland interior. Our findings demonstrates that differetiated neurons doesnot belongs to a particular compartment. it belongs to one or two compartments within PSC. Previous observations from our labortary suggests that cells in PSC are heterogenous in nature. Future studies need to be done to reveal insights into heterogenity and thus mechanisms behind mesodermal to ectodermal transition.

One important caveat of this study is to determine which kind of cellular transition is happening during this mesodermal to ectodermal transition. Is it a transdifferentiation process in which a differentiated cell is directly converted to another differentiated cell without reverting back to pluripotent state or it involves a de-differentiation / reprogramming back to pluripotency and then differentiation? To ascertain this we have to look for the expression of pluripotency markers in PSC of larval lymph gland in which scute is overexpressed. This analysis should be done at intermediate stages of larval development. However, given the timeline in which the cell fate alteration is evidenced, in all likelihood the cells of the niche are undergoing transdifferentiation.

Bibliography:

1. C. H. Waddington. (1957). The strategy of the genes; a Discussion of some aspects of theoretical biology. ***London : Allen & Unwin.***

2. Gurdon J.B., Elsdale Tr, Fischberq M. (1958). Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. ***Nature***. 82, 64-65.

3. Wilmut I, Schnieke AE, McWhir J, Kind A J, Campbell KH. (1997). Viable offspring derived from fetal and adult mammalian cells. ***Nature***. 385, 810-813.

4. Kazutoshi Takahashi, Shinya Yamanaka. (2006). Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. ***Cell***. 126, 4, 663-676.

5. Masako Tada, Yousuke Takahama , Kuniya Abe, Norio Nakatsuji, Takashi Tada. (2001). Nuclear reprogramming of somatic cells by invitro hybridization with ES cells. ***Current Biology.*** 11, 19, 1553-1558.

6. Kazuto and Shinya Yamanakashi Takashashi. (2016). A decade of transcription factor-mediated reprogramming to pluripotency. ***Nature Reviews Molecular Cell Biology***. 17, 183–193.

7. Davis RL, Weintraub H, Lassar AB. (1987). Expression of a single transfected cDNA converts fibroblasts to myoblasts. ***Cell***. 51, 987-1000.

8. Laiosa CV, Stadfeld M, Xie H, de Andres -Aguayo L, Graf T. (2006). Reprogramming of committed T cell progenitors to macrophages and dendritic cells by C/ EBPalpha and PU.1 transcription factors. ***Immunity***. 25, 731-744

9. Zhou H, ,Brown J, Kanarek A, rajagopal J, Melton DA. (2008). In-vivo reprogramming of adult pancreatic exocrine cells to beta cells ***Nature.*** 455, 627-632.

10. Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernigm. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. ***Nature***. 463, 1035-1041

11. Leda m, fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defied factors. ***Cell*** . 142, 375-386.

12. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. (2008). In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. ***Nature*.** 455, 627–632.

13. Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, Wang X, Hui L. (2011). Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. ***Nature.*** 475, 386-389.

14. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE, Herlyn M. (2005). A tumorogenic subpopulation with stem cell properties in melanomas. ***Cancer Research***. 65, 9328-9337.

15. Morghan T. H. 1910. Sex- limited inheritance in *Drosophila.* ***Science*** . 32, 120-122

16. Adams M. D., Celniker S. E., Holt R. A., Evans C. A., Gocayne J. D. (2000). The genome sequence of *Drosophila melanogaster*. ***Science*.** 287. 2185–2195

17. Reiter LT, Potocki L, Chien S, Gribskov M, Bier E.(2001). A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. ***Genome Research***.11, 114-125.

18. Greenspan R. J. (1997). Fly Pushing: The Theory and Practice of *Drosophila* genetics, ***Cold Spring Harbor Laboratory Press, Plainview, NY***.

19. Ashburner M. (1989). *Drosophila*: A Laboratory Handbook, ***Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY***.

20. Simcox, A. A., & Sang, J. H. (1983). When does determination occur in *Drosophila* embryos? ***Developmental Biology.*** 97, 212–221

21. Fristrom, D., Fristrom, J. (1993). The metamorphic development of the adult epidermis**.** ***Cold Spring Harbor: Cold Spring Harbor Press*.** 843–897

22. Gehring, W. J., & Schubiger, G. (1975). Expression of homeotic mutations in duplicated and regenerated antennae of *Drosophila melanogaster*. ***Journal of Embryology and Experimental Morphology.*** 33 , 459–469

23. E. Hadorn. (1963). Differentiation of repeated fragmented sections of male genital discs of Drosophila melanogaster after cultute in vivo . ***Developmental Biology***. 7, 617-629.

24. W.J. Gehring, G. Schubiger. (1975). Expression of homeotic mutations in duplicated and regenerated antennae of *Drosophila melanogaster*. .***Journal of Embryology and Experimental Morphology.*** ,33 , 459-469.

25. S. Schneuwly, R. Klemenz, W.J. Gehring. (1987). Redesigning the body plan of *Drosophila* by ectopic expression of the homoeotic gene Antennapedia. ***Nature***, 325, 816-818

26. G. Halder, P. Callaerts, Gehring WJ. (1995). Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. ***Science***, 267, 1788-1792

27.J. Kim, A. Sebring, J.J. Esch, M.E. Kraus, K.Vorwerk, J. Magee, S.B. Carroll.(1996). Integration of positional signals and regulation of wing formation and identity by *Drosophila* vestigial gene. ***Nature***, 382, 133-138

28. K. Blochlinger, L.Y. Jan, Y.N. Jan. (1991). Transformation of sensory organ identity by ectopic expression of Cut in *Drosophila*. ***Genes Development***, 5, 1124.

29. Rugendorff, A.E, Younossi-Hartenstein, A, and Hartenstein, V.Rouxs. (1994). Embryonic origin and differentiation of the *Drosophila* heart. ***Developmental Biology***, 203: 266–280

30. Mandal, L., Banerjee, U., Hartenstein, V. (2004). Evidence for a fruit fly hemangioblast and similarities between lymph-gland hematopoiesis in fruit fly and mammal aorta-gonadal-mesonephros mesoderm. ***Nature Genetics.*** 36(9): 1019-1023.

31**.** Jung, S.H., Evans, C.J., Uemura, C., Banerjee, U. (2005). The *Drosophila* lymph gland as a developmental model of hematopoiesis. ***Development***. 132(11): 2521-2533.

32. Mandal, L., Martinez-Agosto, J.A., Evans, C.J., Hartenstein, V., Banerjee, U. (2007). A Hedgehog- and Antennapedia-dependent niche maintains *Drosophila* hematopoietic precursors. ***Nature.*** 446(7133), 320-324.

33. Hartenstein V, Campos-Ortega JA. (1984). Early neurogenesis in wild-type *Drosophila melanogaster*. *Developmental Biology*.193, 308–325.

34. Younossi-Hartenstein A, Hartenstein V. (1997). Pattern, time of birth, and morphogenesis of sensillum progenitors in *Drosophila*. ***Microscopy Research and Technique***. 39(6):479-912)

35. Cabrera, C. V. & Alonso, M. C.(1991). Transcriptional activation by heterodimers of the achaete-scute and daughterless gene products of *Drosophila*. ***EMBO Journal***. 10, 2965–2973

36. Murre, C. (1989). Interactions between heterologous helix–loop–helix proteins generate complexes that bind specifically to a common DNA sequence. ***Cell*** 58, 537–544.

37. Gonzalez F, Romani S, Cubas P, Modelell J, Campuzano S (1989). Molecular analysis of the asense gene, a member of the achaete- scute complex of Drosophila melanogaster and its role in optic lobe development. ***The EMBO Journal***. 8, 3553-62.

38. Garcia-Bellido A. (1979). Genetic analysis of the achaete-scute system of *Drosophila melanogaster*. ***Genetics*** 91, 491–520.

39. Jimenez, F. & Campos-Ortega, J. A.(1990). Defective neuroblast commitment in mutants of the achaete-scute complex and adjacent genes of *D. melanogaster*. ***Neuron*** 5, 81–89.

40. Jarman, A. P. (1993). The regulation and function of the helix–loop–helix gene, asense, in *Drosophila* neural precursors. ***Development*** 119, 19–29.

41. Dominguez, M. & Campuzano, S. (1993). asense, a member of the *Drosophila* achaete-scute complex, is a proneural and neural differentiation gene. ***EMBO Journal***. 12, 2049–2060.

42. Cubas, P., de Celis, J.F., Campuzano, S., Modolell, J. (1991). Proneural clusters of achaete-scute expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. ***Genes and Development.*** 5, 996-1008**.**

43. Skeath JB, Caroll SB.,(1992). Regulation of proneural gene expression and cell fate during neuroblast segregation in the *Drosophila* embryo. ***Development.*** 114, 936-946.

44. Artavanis-Tsakonas S, Rand MD, Lake RJ. (1999)**.** Notch signaling: cell fate control and signal integration in development**.** ***Science***. 284(5415), 770-6.

45. Heitzler, P., Simpson, P. (1993). Altered epidermal growth factor-like sequences provide evidence for a role of Notch as a receptor in cell fate decisions. ***Development*** 117(3): 1113-1123.

46. Hombría JC, Brown S, Häder S, Zeidler MP. (2005). Characterisation of Upd2, a *Drosophila* JAK/STAT pathway ligand. ***Developmental Biology***. 288(2), 420-33.

47. Hummel T, Krukkert K, Roos J, Davis G, Klämbt C. (2000). *Drosophila* Futsch/22C10 is a MAP1B-like protein required for dendritic and axonal development***. Neuron.*** 26, 357-370.

48. Varjosalo M, Li SP, Taipale J. (2005). Divergence of hedgehog signal transduction mechanism between *Drosophila* and mammals. ***Developmental Cell***.10, 177-186.

49. Dessaud E, McMahon AP, Briscoe J. (2008). Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. ***Development***. 135, 2489-2509.

50. Roignant JY, Treisman JE. (2009). Pattern formation in the *Drosophila* eye disc. ***Developmental Biology***., 53: 795-804.

51. Durbeej M. (2010). Laminins. ***Cell Tissue Research Journal***. 339, 259-68.

52. Laurila P, Leivo I. (1993). Basement membrane and interstitial matrix components form separate matrices in heterokaryons of PYS-2 cells and fibroblasts. ***Journal of Cell Science***. 104, 59-682.

53. Melina Grigorian, Ting Liu, Utpal Banerjee, and Volker Hartenstein. (2013). The proteoglycan Trol controls proliferation and differentiation of blood progenitors in the *Drosophila* lymph gland. ***Developmental Biology***. 384(2), 301-312

54. Antonio García-Bellido, Jose F. de Celis. (2009). Complex Tale of the *achaete–scute* Complex: A Paradigmatic Case in the Analysis of Gene Organization and Function during Development. ***GENETICS***. 182, 631-639.

55. Taneyhill LA (2008). To adhere or not to adhere: the role of Cadherins in neural crest development. ***Cell Adhesion and Migration***. 2. 223–230.

56.Van. Roy F, Berx G (2008). The cell-cell adhesion molecule E-cadherin. ***Cell Molecular Life Sciences***.65, 3756–3788.

57. Nelly Auersperg, Jie Pan, Bryon D. Grove, Todd Peterson, Janet Fisher, Sarah Maines-Bandiera, Aruna Somasiri and Calvin D. Roskelley.(1999). E-cadherin induces

mesenchymal-to-epithelial transition in human ovarian surface epithelium. ***PNAS.*** 96 (11), 6249-6254.

58..RonghuiLi[12]JialiangLiang[12]SuNi[1]TingZhou[1]XiaobingQing[1]HuapengLi[1]WenzhiHe[1]Jiekai Chen[1]FengLi[1]QiangZhuang[1]BaomingQin[1]JianyongXu[1]WenLi[1]JiayinYang[1]YiGan[1]DajiangQi n[1]ShipengFeng[1]HongSong[1]DongshanYang[1]BiliangZhang[1]LingwenZeng[1]LiangxueLai[1]Migue l,AngelEsteban[1]DuanqingPei. (2010). A Mesenchymal-to-Epithelial Transition Initiates and is required for the Nuclear Reprogramming of Mouse Fibroblasts. ***Cell Stem Cell.*** 7(1):51-63.

59. Thiery JP (2002). Epithelial - mesenchymal transitions in tumour progression. ***Nature Review Cancer***.2, 442-54

60. Chaffer CL, Brennan JP, Slavin JL, Blick T, Thompson EW, Williams ED. (2017). Mesenchymal-to-epithelial transition facilitates bladder cancer metastasis: role of fibroblast growth factor receptor-2. ***Cancer Research***.66, 11271-11280.

61. Timothy R. Jackson, Hye Young Kim, Uma L. Balakrishnan, Carsten Stuckenholz, Lance A. Davidson. (2017). Spatiotemporally Controlled Mechanical Cues Drive Progenitor Mesenchymal-to-Epithelial Transition Enabling Proper Heart Formation and Function. ***Current Biology.*** 27,1326-1335.

62. Domínguez M , Hafen E. (1997). Hedgehog directly controls initiation and propagation of retinal differentiation in the Drosophila eye. ***Genes Development***. 11, 3254-3264.

63. Yoo YA, Kang MH, Lee HJ, Kim BH, Park JK, Kim HK, Kim JS, Oh SC. (2011). Sonic hedgehog pathway promotes metastasis and lymphangiogenesis via activation of Akt, EMT, and MMP-9 pathway in gastric cancer. ***Cancer Research.*** 71, 7061-7070

64. K D McClure, G Schubiger. (2007). Transdetermination: Drosophila imaginal disc cells exhibit stem cell like potency. ***International Journal for Biochemistry and Cell Biology***. 39, 1105-1118