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Neuropathological aspects in axonal growth development in Drosophila

MASTER THESIS

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submitted by

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My external supervisor from Harvard University in Boston, Massachusetts, USA was Professor David Van Vactor from the Department of Cell Biology, Harvard Medical School.

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Contents

Research Hypothesis:

Translational Regulation is involved in microtubule +Tip Function during Axon Guidance in Drosophila melanogaster.

1) Introduction:

Cells in the nervous system have a very complex way to communicate with each other as well as with their environment (COHEN, 2005). In highly developed species, growth and organ formation are only accomplished by minute and precise cell signaling pathways (BAAS, 1997). Cell signaling is a complex thus vital way of cells to form tissues, organs and finally create organisms. During development of the nervous system, neurons project axons over long distances (YU et al., 2001). The study of axonal growth cone processes in the developing central nervous system includes the detection of molecules that provide guidance cues for growth cone orientation (KALIL, 2005; MISRA, 2002). Axon guidance requires coordinated remodeling of actin and microtubule polymers (LEE, et al., 2004). There has to be a very precise way of guiding the leading edges along the track to find their final target sites. These so called guidance cues can be either attractants or repellents (LOWERY, 2009). This guidance cue bifunctionality depends on a variety of factors including the intracellular state of the growth cone, differential expression of receptor complexes, and cross talk between intracellular signaling cascades (LOWERY et al., 2009). All those above mentioned factors together coordinate the proper and precise guiding of axons in nerve cells as well as guide growth cones in the central nervous system in Drosophila.

In neural tissue the Abl- CLASP pathway is involved in embryological growth cone formation and navigation (LEE, et al., 2004). CLASP, a microtubule-associated plus-end tracking protein acting as a partner of the Abelson (Abl) tyrosine kinase, is suspected to be a key conductor in cell regulation (WILLS, 1999). In a recent model CLASP earned more attention as a regulator in both microtubule and actin networks coordinating also growth cone steering in nerve cells during central nervous system development and embryogenesis (LOWERY, LA., 2009 and 2010).

In Drosophila a forward genetic screen was used as a tool that unveiled certain molecular factors that interact with CLASP (LOWERY, LA., 2010). One of the factors that showed up in a screen belong to the eukaryotic translation regulation group abbreviated as eIF factors in particular eIF4E pulled down in the screen. Thus an interaction between eIF4 translational factors and Orbit/CLASP is discussed to be involved in axon guidance and central nervous system development (MORESCO, 2005). This novel finding connects Orbit/CLASP with translation/RNA regulation factors of the eukaryotic initiation factor family and triggers deeper research interest in how far translation regulation plays a role in actin and microtubule-associated protein interaction especially in nerve cells. Recent literature points towards Orbit/CLASP being an important player in the microtubule-actin cross-talk interaction (LOWERY, LA., 2010). Orbit/CLASP protein localizes to *Drosophila* growth cones (LEE, et al., 2004).

Specific aims and objectives at the Department of Cell Biology:

The aim of this work is to investigate cellular pathways in regard to the key role of CLASP. One of the primary goals is to define and confirm potential genetic pathways of interaction between TACC, CLASP and the eIF translation factor family group. The outcome of a genetic and proteomic screen unveiled several interactions between different cell signaling factors. The resulting interactome needs further confirmation and careful study as the most promising hits from the screen could uncover new cellular pathways in order to understand neural growth cone motility better. The interactome showed several pathways in cells that overlap and where interactors could be broken into several categories: the largest was cytoskeletal regulators, which include two actin-binding proteins, two Microtubule +TIPs, and several proteins that could bind to both Microtubule and actin. Both Microtubule and actin are crucial for neural development so further elaboration on interacting factors is researched.

In my project the aim one is to analyze whether loss of function mutants lacking TACC, a transforming acidic coiled-coil protein factor may cause axon guidance effects in the embryonic CNS.

Aim two is to study the network elaboration between CLASP, TACC and various translation initiation factors. The goal is to work out specific interaction traits between CLASP and translational players like eIF4E. Hence CLASP is considered to be a Microtubule +TIP, also has actin binding sites and is moreover known to bind actin in non-neuronal systems thus being responsible for cell motility. Using the phenotypic expression in the fly's eye I want to study the role of TACC and eIF4E on CLASP.

Aim three is to investigate the importance of TACC- eIF4E binding site in drosophila. Is the TACC- eIF4E binding site important in context of nerve cell growth?

In this work Drosophila melanogaster is used to investigate molecular pathways to uncover how certain signals are processed in cells. Neural tissue in this context is highly interesting as to study axonal guidance in developing drosophila. During nervous system development precise axon guidance is an essential yet highly integrative process that leaves many questions and issues unanswered. Understanding how abnormalities in neuronal development arise as well as the mechanisms by which axons form complex functional neuronal networks are still poorly grasped. On top of that rests the hope to establish nerve regeneration therapeutics. A major quest in this research field is to shed light in the logic by which guidance information is integrated at the level of cytoskeletal dynamics control during axon path finding. My task during this research project was to investigate how TACC and several translation initiation factors (eIFs) influences CLASP in neural development.

Figure 1: Schematic view of a wildtype (wt) Drosophila melanogaster. Bookcover from GREENSPAN, J. R. (1997): Fly Pushing. The Theory and Practice of Drosophila Genetics. Cold Spring Harbor Laboratory Press.

FlyBase a database for genetic researchers:

FlyBase (http://flybase.org) is the primary database of integrated genetic and genomic data about the Drosophilidae, of which Drosophila melanogaster is the most extensively studied species. The flybase website houses information about the structure and function of the Drosophila

genome. More than 55,000 gene records from over 500 species of Drosophila accommodate data regarding gene function and expression patterns, and phenotypes and genetic interactions owing to mutant alleles of those genes (http://flybase.org). Ordering stocks and the availability of stocks is linked to allele, chromosome aberration, as well as transgenic insertion records. The FlyBase home page (http://flybase.org) harbors a whole lot of pull-down menus in the header panel. Among the most useful might be the menu point resources as it provides information on external links along with links to several stock centers.

Developmental life-cycle stages in Drosophila:

Under standard laboratory conditions the whole life cycle does not take longer than some 10 days. Embryogenesis occurs within the egg that is deposited into the food, and after slightly less than 24 h, the first instar larva hatches. During the next four days the larva takes up its main task namely feeding. This growth period includes two molts. In these four days the larva increases roughly 200-fold in weight. This amazing mass accumulation is aided by the endoreplication of larval tissues. This is tissue that will be destroyed during metamorphosis and will not contribute to the adult fly. In contrast, the so-called imaginal discs consist of diploid cells and during metamorphosis will be transformed into the adult body structures. Toward the end of the third larva instar; this is about the fifth day after egg deposition, the larva stops feeding and leaves the food. This is referred as the wandering stage. The larva is in search of a dry place suited for pupariation. Metamorphosis takes place in the pupa case during the following four days, and some 3 mm in length with females slightly larger than males. Both environmental condition and genetic makeup impact on body size and also weight (MACDONALD, 2001).

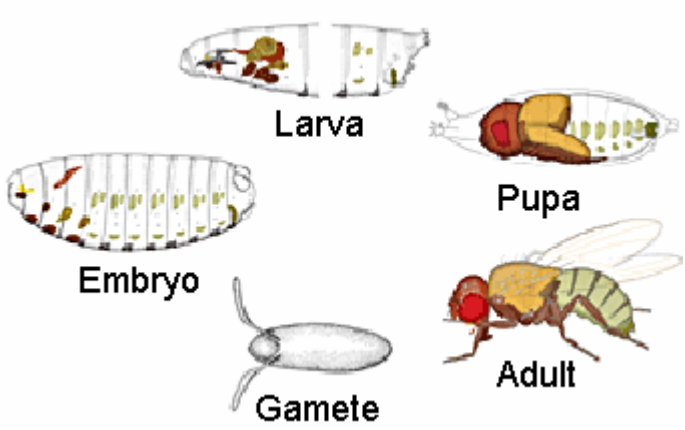


Figure 2: The cycle of drosophila development lasts about two weeks. Starting form the gamete and egg via embryo to three instar larva stages and pupa until the final stage: the adult fruit fly. Source: http://flybase.org/static_pages/imagebrowser/imagebrowser10.html

The females are already receptive less than 12 hours after eclosion, and they start to lay eggs soon after mating. Therefore, two weeks usually suffice for each generation in a crossing scheme. The egg production for each female reaches up to 100 eggs per day with a fecundity peak between day 4 and day 15 after eclosion.

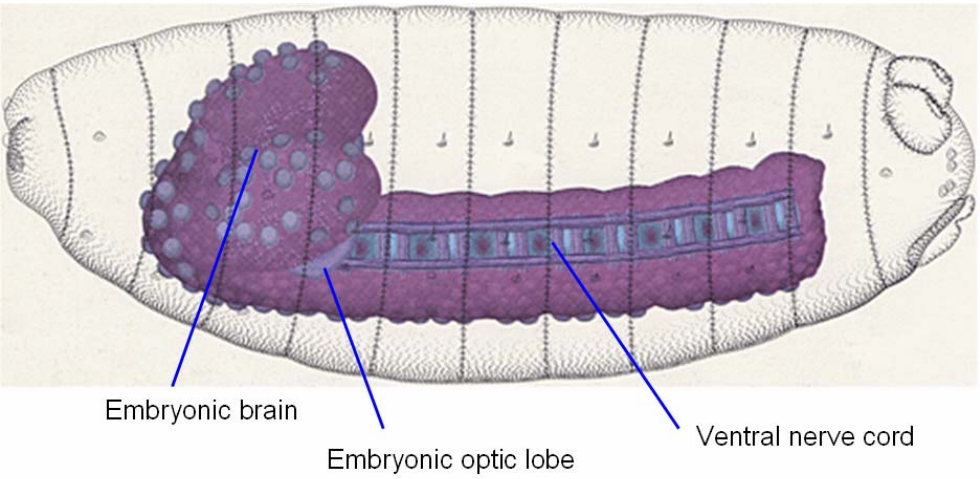


Figure 3: Image of a drosophila fly embryo in stage 17 during development. These embryos are specifically stained with dab that is specific for nerve cells. At stage 17, the deep indentations in the lateral margins of the ventral nerve cord (vg) that had separated individual neuromeres have disappeared. The CNS becomes invested with a sheet of perineurial cells. Source: http://flybase.org/reports/FBim0000650.html

Fly husbandry and crossing flies:

Fly breeding is best performed at room temperature as not many technical lab equipment is needed (i.e., incubators). The best conditions for fly raising are at 25°C, 60% relative humidity. At 25°C the generation time of the flies is fastest and the flies have the best viability. Under these conditions the generation time from egg to emerged adult ranges between 9 and 11 days depending on the breed. Flies can also be kept at higher or lower temperatures within a range from about 18°C to ca. 29°C.
In order to prevent mold and mites it is advisable to flip those flies every two weeks into new vials. At maximum the flies should only be kept for 18 days in the same vial as after this time one can mistake the second-generation progeny for their parents; since the parents have not been separated (GREENSPAN, J. R.: Fly Pushing. The Theory and Practice of Drosophila Genetics).

Stocks that are only for breeding and need no visual determination can be transferred without anesthetization. Tapping the flies down to the bottom of the old vial without drowning them in their food, then placing a new vial on top of the old, flip them over and tap the flies down into their new home is easy to learn. Stray flies can be an issue, especially when you lack the practical hands on working in fly pushing but it’s easy to handle as fly traps are straightforward in maintenance and set up. How many flies are needed to set up a new stock depends very much on the fertility of the stock which can be impaired or significantly reduced due to the number of genetic modifications. This also has to be taken into account when it comes to the question of how many females are required to start up a new stock. Soggy cultures from the cumulative waste products as well as the incapability of determining wing phenotype as wings will stick to the flies bodies are the two major problems that arise when vial fly population is too dense. The best way to compensate for not knowing exactly how many flies to start with is to watch the culture as it develops. When the food starts to look churned up there are enough larvae and it’s time to dump out the parents. This can take

roughly four days, depending on the genotypes (GREENSPAN, J. R. 1997; STOCKER, 2008).

The molecular ingredience –balancers in fly genetics:

The Drosophila genome comprises four chromosomes. Many balancer genes exist for the X, second, and third chromosomes. For the fourth chromosome balancers are not necessary due to the fact that there is no exchange occurring during mitosis thus no recombination (GREENSPAN, J. R., 1997).

Balancer genes in flies allow quick discovery of genetic modifications in flies. Fly genetics basically focuses on the ability to perform crosses in which each possible genotype in the progeny is recognized easily and unambiguously.

It is moreover important to have virgin females rather than from stray flies or from unwanted pregnancies. A unique characteristic of fruit fly female is their ability to store male sperm that makes it necessary to collect virgin females as to secure progeny of only the wanted genotype. In many cases due to balancer genes the population of flies may not be the healthiest among the progeny thus it is advisable to start working with a fairly large population of flies.

Balancers prevent crossing over during the course of genetic recombination between homologous chromosomes during meiosis. On the genetic level balancer chromosomes prevent crossing over because they are the products of multiple, nested chromosomal inversions so that synapsis between homologous chromosomes is disrupted. Without balancers there would be several completely different allele combinations after a couple of generations.

There are three characteristics that make balancer chromosomes unique in Drosophila genetic research: They suppress recombination with their homolog, carry dominant markers and negatively affect reproductive fitness when carried homozygously. By suppressing reproductive fitness when

carried homozygously a balancer chromosome ensures that the population it is carried in does not become fixed for the balancer chromosome. Suppression of the homologous chromosome is crucial as this preserves the genotype over the course of generation in the interested allele.

If crossing over between a balancer chromosome and the balancer's homolog does occur during meiosis each chromatid ends up lacking some genes and carrying two copies of other genes. Recombination in inverted regions leads to dicentric or acentric chromosomes. This means in such recombinant events chromosomes occur with either two centromeres or no centromere. Progeny carrying chromosomes that are the products of recombination between balancer and normal chromosomes are not viable. Balancer genes attached to dominant marker genes visualize genotypes that would not be able to be detected without the help of balancer genes in the fruit fly. Those dominant markers can be green fluorescent protein or enzymes that allow for easy segregation analysis. Since the discovery of balancer genes the method has been elaborated that multiply chromosomes are highly unlikely to undergo exchange with their normal homologs.
Among the balancer genes used during my experiments were TM3 that causes short bristles, CYO that makes curled wings in flies, TM6B that carries a dominant allele of Antennapedia.

The GAL4 System:

The GAL4 system is a universal tool for the expression of genes in drosophila. It is a system that requires two lines of flies. One of the lines is a transgenic line, the driver, expresses GAL4 in a known temporal or spatial pattern and the second transgenic line, the responder, contains a UAS-dependent transgene (BRAND, et al. 1993). The GAL4 system requires an appropriate expression pattern. Ectopic gene expression is important in obtaining functional data on a large number of genes.
A large number of lines can be generated and screened for the GAL4-expression pattern to obtain drivers in the tissue of interest. This interesting tool was first accomplished by adapting the enhancer-detection technique

(BELLEN et al., 1989; O ´KANE et al., 1987) a vector was constructed, pGawB in which GAL4 is under the control of a weak P-transposase promoter. This P-transposases makes random insertions in the genome at several sites. GAL4 depends on the regulatory elements surrounding the integration site of the vector.

Originally GAL4 was discovered as a transcriptional regulation factor in yeast. GAL4 is an archetypal eukaryotic transcription factor isolated as an activator of genes responsible in the metabolism for galactose in yeast. GAL4 encodes a protein of 881 amino acids, identified in the yeast *Saccharomyces cerevisiae* as a regulator of genes (e.g., *GAL10* and *GAL1*) induced by galactose (LAUGHON *et al.*, 1984b; LAUGHON and GESTELAND, 1984a; OSHIMA, 1982). Due to its high level of conservation in the eukaryotic transcription machinery it has become a widely used and popular tool to study the function of genes thoughout species. Many studies have been performed using Saccharomyces cerevisiae as model organisms (WEBSTER, et al., 1988; KAKIDANI, et al., 1988; MA, et al., 1988).
In a number of notable studies on transcriptional regulation, the DNA binding and transcriptional activation functions of GAL4 were identified, demonstrated to be separable, and meticulously defined (PTASHNE, 1988). GAL4 regulates the transcription of the divergently transcribed *GAL10* and *GAL1* genes by directly binding to four related 17 basepair (bp) sites located between these loci (GINIGER *et al.*, 1985). These sites define an Upstream Activating Sequences (UAS) element, analogous to an enhancer element defined in multicellular eukaryotes, which is essential for the transcriptional activation of these GAL4-regulated genes.
In 1988 Fischer *et al.* demonstrated that GAL4 expression was capable of stimulating transcription of a reporter gene under UAS control in *Drosophila.* This activity is not limited to *Drosophila*, as GAL4 can function in a wide variety of systems to activate transcription from the UAS element (KAKIDANI et al., 1988; MA *et al.*, 1988; WEBSTER et al., 1988).
Targeting gene expression in a temporal and spatial fashion has proven to be one of the most powerful techniques for addressing gene function in vivo (BRAND et al., 1994). Thus, the spatial and temporal control of GAL4

expression is based on endogenous enhancers. GAL4 under the P-transposase promoter creates almost 7000 GAL4 drivers that are documented and available online at the GAL4 Enhancer Trap Data Base (HAYASHI, 2002).

In 1994 Brand and Perrimon published a bipartite approach for directing gene expression in vivo. In this system, expression of the gene of interest, the responder, is controlled by the presence of the UAS element, in this case five tandemly arrayed and optimized GAL4 binding sites. Because transcription of the responder requires the presence of GAL4, the absence of GAL4 in the responder lines maintains themin a transcriptionally silent state. To activate their transcription, responder lines are mated to flies expressing GAL4 in a particular pattern, termed the driver. The resulting progeny then express the responder in a transcriptional pattern that reflects the GAL4 pattern of the respective driver.

This bipartite approach, in which the two components of the system, the responder and the driver, are maintained as separate parental lines, has numerous strengths (DUFFY, 2002). First, the transcriptional inactivity of the parental responder line means that transgenic responder lines can be generated for gene products that are toxic, lethal, or have reduced viability when expressed. When crossed to a GAL4 driver, the production of a gene product is triggered and results in a reaction int he responder line, thereby causing cell death, lethality, or reduced viability. This provides a powerful tool to study the effect of loss of specific cells on the process of interest, as well as the function of these toxic, lethal, or oncogenic genes. For example, several responder lines currently exist for genes such as reaper that can trigger programmed cell death (APLIN and KAUFMAN, 1998; BRAND and PERRIMON, 1994; ZHOU *et al.*, 1997).

An additional strength of the system arises from the ability to target expression of any responder in a variety of spatial and temporal fashions by mating it with distinct GAL4 drivers (O`KANE, 1987). In addition with the advent of RNA interference (RNAi) technology it is now possible for the geneticist to have a handle on a large selection of tools with which to drive

expression.

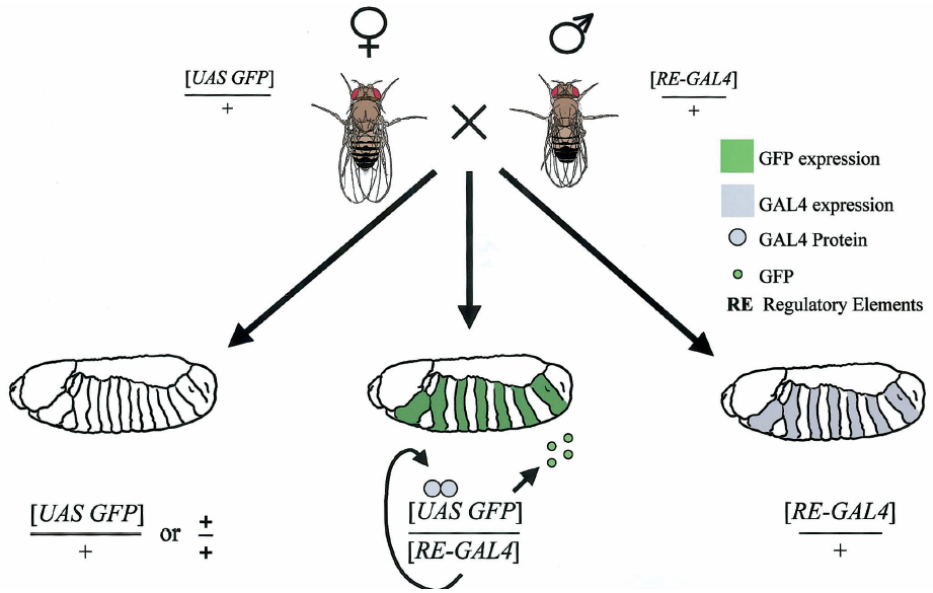


Figure 4: The bipartite UAS/GAL4 system in Drosophila. This is the case when females carrying a UAS responder (UAS-GFP), mate with males carrying a GAL4 driver. The resulting progeny containing both elements of the system are produced. The presence of GAL4 in an altering segmental pattern in the depicted embryos then drives expression of the UAS responder gene in a corresponding pattern. In this example GFP is expressed.
Picture from: DUFFY et al. (2002): GAL4 System in Drosophila A fly Geneticist's Swiss Army Knife.

As the GAL4 driver and the UAS responder are separated in two different lines this allows analysing a single UAS-dependant transgene in multiple tissues and/or time-points trough the use of different drivers. One can also create a transgenic encoding for a lethal product because the UAS construct will not be expressed in the absence of GAL4. The progeny of such flies then show the lethal phenotype.

The rescue of a mutant phenotype:

Furthermore, rescue experiments can be performed by re-establishing the endogenous gene function with the appropriate driver in a mutant background. In some experimental settings the mutant background lacks the expression of the gene studied and the phenotype is significantly mortal, so a cross to a GAL4 expression line that provenly has expressed the gene product rescues the mutant phenotype.

In a rescue experiment a severe gain of function or loss of function phenotype can be brought back to a wild- type appearing phenotype when crossed to a mutation in a fly that has a wild- type copy of the gene.

There are many modifiers where duplications and deletions have modifying effects. Commonly a deletion acts as a dominant suppressor and duplications act as a dominant enhancers. This variegation is referred to as class I position- effect variegation modifiers. It is important to be aware of these effects when modifying genes as gain of function as well as loss of function genotypes will influence the severity of the resulting phenotype. This is the how a genetic interaction can be elicited based on rescuing a phenotype.

In wild type modifier genes the dose of phenotype changes is not dramatic. However there is a model existing (LOCKE et al., 1988) for class I position-effects that modify transcription in a way that heteromultimeric complexes are coded by many adjacent loci. In detail, there are four different proteins forming a regulatory complex known to correlate with enhancement or suppression (ASHBURNER, 2005). This means if position- effect deletions or duplications occur an extreme effect of a specific phenotype can be observed. That is to blame on the position-effect variegation not on the initial gene modification studied.

The rescue of such a mutant phenotype is when introducing a transposon construct bearing a wild-type copy of a candidate gene and showing that the gene carried by the rescue construct, is indeed the same as that defined by the mutation.

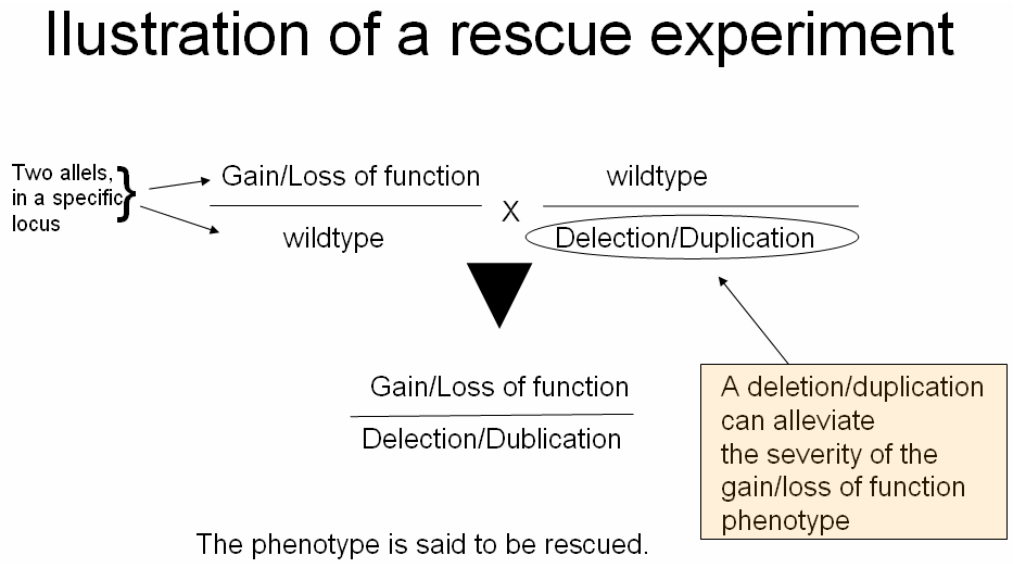


Figure 5: This is an example of a rescue experiment to illustrate how severe gain or loss of function phenotypes can be rescued by crossing them to a wildtype copy with a deletion or duplication.

Nerve growth cones and guidance structure models:

In every mammalian cell actin and microtubule polymeres form a scaffold that brings the integrity of each individual cell (TESSIER-LAVIGNE, 1996). In nerve tissue growth happens at so called neural cones (KENNETH, 2002). At the leading edge of a growing axon is the neural growth cone. This is a structure highly sensitive to rapidly responding to its environment (PATEL, 2002). The anatomy of a growth cone is made up of lamellipodia, which contain cross-linked networks of actin filaments and filopodia, tensile structures composed of bundled F-actin that probe the extracellular environment (GERALDA, 2008). This peripheral actin network is associated in the proximal portion of the growth cone with microtubules situated in the distal part of the axon shaft and contributes to the assembly and translocation of microtubules into more proximal regions of the growth cone (MALLAVARAPU and MITCHISON 1984; MITCHISON, 1988; SUTER et al., 2000).

These growth cones react to attractive and repellent factors (DICKSON, 2002; LOWERY, 2010). According to the molecular composition of protein expression axonal cones are guided to grow or terminate growth.

The growth cone basically advances in three steps: protrusion, engorgement and consolidation (see Figure 2). The growth cone is the very tip of an axon that responds to molecular cues (TANI, 2005). These cues can be adhesive molecules that provide the proper environment to make the axon move along the cellular tissue track (AKHMANOVA, 2008). Cells can express adhesive molecules, or may have built in molecules into the extracellular matrix (ECM). At the interior of the cell additional molecular pathways are switched on to keep the neural fibres growing (POLLARD, 1986).

Although cell diversification is largely complete at or shortly after birth, organs must possess a mechanism to replenish cells as they die, either by natural wear and tear called homeostasis, or by injury (FUCHS, et al. 2004). To accomplish this feat in the adult world, many developing tissues set aside life-long reservoirs of somatic stem cells, which retain some of the versatile

characteristics of their early embryonic stem cell properties (FUCHS, et al. 2004). Nerve cells were long thought not to have self- renewable capacities are now also strongly suspected to show differentiated mechanisms to maintain axonal growth throughout their entire lifespan (FUCHS, et al. 2004).

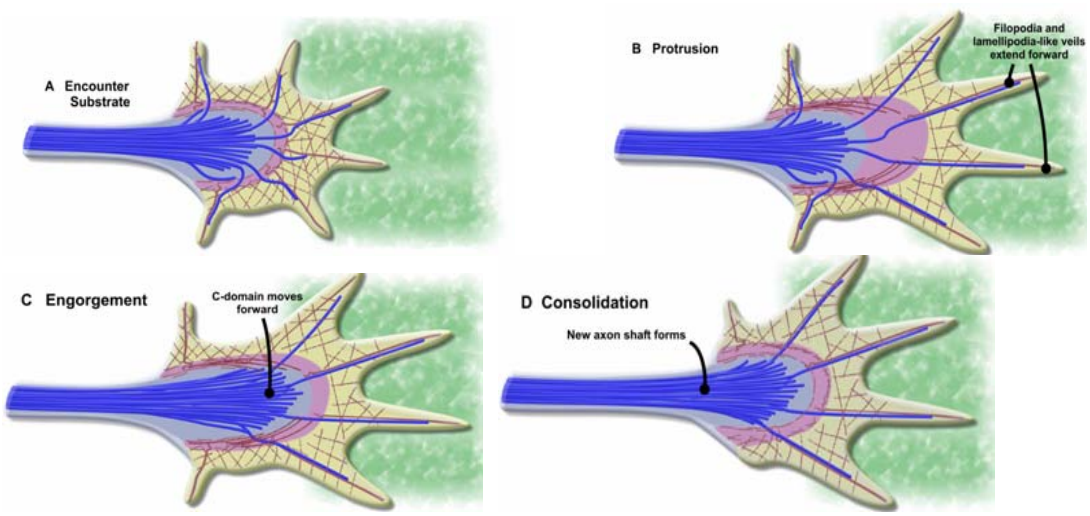


Figure 6: The different stages of axon guidance in nerve cells comprise of encountering substrate, protrusion, engorgement and consolidation. Source: LOWERY, Laura-Anne 2010.

At the tip of an axon the growth cone leads an elongation process. The growth cone is a highly dynamic structure, confirmed in live studies (RAMON, Y. CAJAL S., 1890; HARRISON, 1907; DENT, 2003). Molecular paths along the track are responsible for the navigation of the growth cone that is steadily elongating the axon by adding new structural components such as microtubules and membrane to its tip (GOLDBERG, 2003). Many species need growth cones that provide guidance cues through actin dynamics in the growth cone periphery (DENT, 2003; KALIL, 2005; MEIMA, 1997; SUTER, 2004; ZHOU, 2004). The peripheral filamentous actin cytoskeleton executes a continuous, myosin-driven retrograde flow that involves ATP-dependent addition of globular actin to actin filaments at their barbed ends, a gradual change of actin-bound ATP to ADP, and hydrolysis or severing at their pointed ends (CARLIER, 1991; DISANZA, 2005;

GUNGABISOON, 2003). This molecular milieu creates an environment for the nerve growth cone to stir directions in tissues. One prominent molecule that directs growth cone steering is netrin (ROUND, 2007; LIVESEY, 1999). The process of actin assembly and disassembly and its organisation into lamellipodial networks and filopodial bundles is regulated by complex molecular machinery (DISANZA, 2005; VIGNJEVIC, 2003; SVITKINA, 2003; FAIX, 2006).

Whereas in migrating cells stress fibres pull the entire cell forward, in neuronal cells usually the cell bodies remain at a place and only the growth cone migrates (TANAKA, 1996; GREENBERG, 2000). Neuronal cells cores stay connected by the steadily elongating axons, as the axonal core contains bundled, stable microtubules acting as pathways for axonal transport (SANCHEZ-SORIANO, 2007). In other tissues and cells actomyosin-containing filopodia and lamellipodia bear a similar mechanism that makes focal contacts that are found at the leading edge of growth cones (KAVERINA, 1998). A dynamic population of microtubules and cell polarity markers at these focal contacts has been revealed such as Par-3 and -6 (SHI, S. 2003; SUTER, 2000; KALIL, 2005; ZHOU, 2004; 2004; RENAUDIN, 1999; ROBLES, 2006).

The dynamics of microtubules and the expression of actin dynamics:

Growth cone guidance is crucially based on actin dynamics (O'DONNELL, 2009; WEN, 2006). On the other hand growth cone advance depends on microtubules (GIGER, 2005). One of the first experiments that revealed the interplay between actin and microtubules showed that axonal growth could be repressed when actin polymerisation blockers are added into the solution (BRAY, 1978; DENT,2003; KAUFMAN, 1998; MITCHINSON, 1984; WU, 1990; YAMADA, 1973;).

In cells microtubules are vital to accurately maintain the cells organization and structural features (CULLEN, 2001; MITCHINSON, 1987). Form and function of microtubules arrays vary in each cell type, and are also

dependant on developmental and cell- cycle phases (CASSIMERIS, 1999; SMALL, 1978 and 1998).

Microtubules in the axon shaft and central zone of growth cones are stable and bundled, whereas single unstable microtubules elongate into and retract from the peripheral actin-rich zone in a highly dynamic fashion (BRATMAN, 2008; GRABHAM, 2007; O'DONNELL, 2009). These microtubules can be trapped or stabilised through signals in the growth cone periphery, thus determining the direction in which microtubules of the axon shaft will extend (DENT, 2003: KALIL, 2005; ZHOU, 2004; SUTER, 2001 and 2004). The microtubule growth and shrinkage mechanism is by its own an autocatalytic process (WANG, 2005; KERSSEMAKERS, 2006; MAHADEVAN, 2005).

Cross-talk between microtubules and actin is an essential aspect of regulation of growth cone behavior and this process has long been known to be facilitated by microtubule-associated proteins (GRIFFITH, 1987). Up to now there are a number of microtubule-associated proteins (MAPs), both motor and nonmotor proteins that are collectively known to regulate microtubule dynamics and organization in the cell (KLINE-SMITH, 2004; KINLEY, 2003). A molecule that plays a role in growth cone formation is for example MAP2 that has the capability to bind both microtubules and actin (DEHMELT, 2005). Among other factors are CLASP, Msps and a cluster of eIF translational control proteins that pulled down in a parallel genetic and proteomic screen (LOWERY, et al., 2010).

There are many other major families of axon guidance cues and their receptors that drive the axon growth cone (BASHAW, et al., 2010; LOWERY, et al., 2010). Among these factors are for example Rho GTPases that orchestrate actin filament assembly and disassembly through the control of actin polymerization, branching, and depolymerization (HUBER et al., 2003; WITTMAN, 2001). Another molecule that is required for growth cone guidance rather than axon extension in *Drosophila* is the highly conserved DPod-1 (ROTHENBERG, 2003).

In *Drosophila*, two molecules with dual actin- and microtubule-binding capabilities have been discovered. Short stop/Kakapo is a member of the

Spectraplakin family of cytoskeletal linker molecules (RöPET, 2003), and its cytoskeletal binding activity is required for axon extension (LEE, 2002).

CLASP as a key player in the cell signalling pathway:

CLASP is a key molecule that belongs to the plus tip cell molecules. It is involved in microtubuline and actin formation and movement. Cytoplasmatic linker proteins (CLIPs) and the CLIP-associating proteins (CLASPs), which are plus-TIPs that have diverse functions in the generation of cellular asymmetry, are discussed to be key regulators in actin and microtubuline cross talk (EFIMOV, 2007; LOWERY, 2010).

There are basically three types of CLASP cytoskeleton proteins that stabilize cells (PEREIRA, 2006).

Intermediate filaments are considered as stable structures in the cell (GALJART, 2005). They do not move and their assembly is based on the association of antiparallel dimers into oligomeres which generates apolar filaments. Actin filaments and microtubules on the other hand make up the other two cytoskeleton types and fall into the category of dynamic cytoskeletal proteins (WITTMAN, 2005). Actin filaments and microtubules are used to sustain and adapt cellular shape whereas in contrast to intermediated filaments that confer strength on a cell. These two properties allow a cell to generate asymmetry (GALJART, 2005; TSVETKOV, 2007).

Translational control and RNA mediation by the eIF group:

A key role in nerve tissue formation depends on the process of mRNA translation and its regulation (CORRAL, 2004). Eukaryotic translation initiation factors, also known as eIF, are proteins that are encoded by the eIF genes. The expression of the eukaryotic initiation factors (eIF) determines the fate of cells in complex cell development. The initiation of the translation of most eukaryotic mRNAs requires the recognition of the cap structure being present at their 5´end (TRACHSEL, 1979). In the context of axon growth and nerve cell development the eIF4F complex works in close conjunction with the 5-prime end of the translational machinery and binds to the 40S ribosomal subunit (O'KANE, 1987). The eIF4F regulatory complex comprises of several molecules that together regulate how and when the cell cycle will be halted or turned on (CASSIMERIS, 1999).

In addition to the eIF4F complex with its various components there are other molecules that regulate the process of mRNA translation and its regulation. eIF2 provided an example for the control of eukaryotic protein synthesis by protein phosphorylation and remains the most intensively studied translation factor. eIF2 is regulated by phosphorylation (FRANKLIN- DUMONT, 2007). In its active GTP-bound form eIF2 binds the methionyl-initiator tRNA and therefore associates with the 40S ribosomal subunit whereas in its inactive GDP-bound form it does not bind to the methionyl-initiator tRNA (HINNEBUSCH, 2000). Lower eukaryotes possess two distinct eIF2 kinases (MAHLMANN.1998). Modulation of eIF2 activity occurs mostly in response to a wide range of stress conditions observed mainly in eukaryotes (PROUD, 2004). Impairment of protein synthesis is linked to an unfavorable environment for the cell and thus for example hampering axon regeneration (GRIMPE, 2002). Thus resulting in a slowing down of the translational machinery or even shutting it completely down. This is accomplished through the phosphorylation of the eIF2- alpha, a subunit of the eIF2B complex (FOGLI, 2006). This holoenzyme complex is discussed to cause

vanishing white matter (VWM) a neurodegenerative disease that is inherited and occurs mostly among young children (KNAAP, 2002; FOGLI, 2006; SCALI, 2006).

The model of eukaryotic translation regulation:

With a few exceptions the initiation site selection in eukaryotes continues to be interpreted in terms of the scanning ribosome model (JACKSON, 1996). The scanning entity according to generally held opinions is the 40S ribosomal subunit with a variety of initiation factors bound to it (JACKSON, 1997). Especially the regulation of translation is conducted primarily at the initiation phase and the most prominent group of regulators comprises the translation factor eIF4 group, that is involved in the recognition of the 5´ prime cap structure. These proteins interact directly with the 5´-untranslated region (UTR) of mRNAs.

In its simplest terms, the process of translation initiation consists of the binding of methionly-initiator tRNA, and the joining of the 60S ribosomal subunit to form the 80S initiation complex (TRACHSEL, 1996). Each of these steps is stimulated by soluble protein factors knows as eukaryotic initiation factors.

Reconstitution of this process in vitro using purified ribosomes and eIFs indicate that binding of Met-tRNA to the 40S subunit is a prerequisite for mRNA binding (BENNE et al. 1978; PAIN, et al. 1983). The Met-tRNA is transferred to the 40S subunit by a ternary complex consisting of Met-tRNA, the heterotrimeric initiation factor 2 (eIF2), and GTP. This whole reaction is stimulated by eIF3, eIF1A, and also most likely by eIF5B (BENNE, et al., 1976; TRACHSEL, 1979). The resulting 43S pre-initiation complex binds mRNA forming the 48S complex. This binding reaction between the 43S and the mRNA- associated factors is promoted by the mRNA-associated factors consisting of eIF4E, eIF4G, eIF4A, eIF4B and the poly [A]-binding protein and also by eIF3 residing the 43S complex (PROUD, 1992).

In this molecular regulatory machine the eIF4F complex comprises of eIF4A and eIF4B; works as the helicase protein complex. In order to unwind the secondary structure at the 5’ untranslated region (UTR) this second

molecular structure made out of eIF4A and eIF4B has to bind to the eIF4F complex and form a so called holoenzyme. This creates a regulatory tool for the cell to promote the landing of the 40S ribosomal subunit and the subsequent search for the initiator codon (GRINGRAS, et al. 1999, HERNANDEZ et al. 2008; ZAPATA et al. 1994). Thus making the eIF4E complex an integral cap-dependent translational factor (ALTMANN, 1985; GINGRAS et al. 1999). Due to its central role in cap-dependant translation, regulation of eIF4E activity is also critical to normal cell growth (HERNANDEZ, 2008). This explains the observations that overexpression of eIF4E results in overgrowth and malignant transformation (de BENEDETTI and GRAFF, 2004; DUA et al. 2001; ROSENWALD, 2004; SONENBERG and GINGRAS, 1998).

Finally there is the eIF1 translational control factor which appears to stimulate the overall process of initiation without playing any well defined role; eIF3, in turn is a high molecular weight complex that has two functions ascribed; namely preventing premature 60S subunit joining and decrease binding to mRNA; and the eIF2/GTP/Mct-tRNAi ternary complex (SEYUN, 2010).

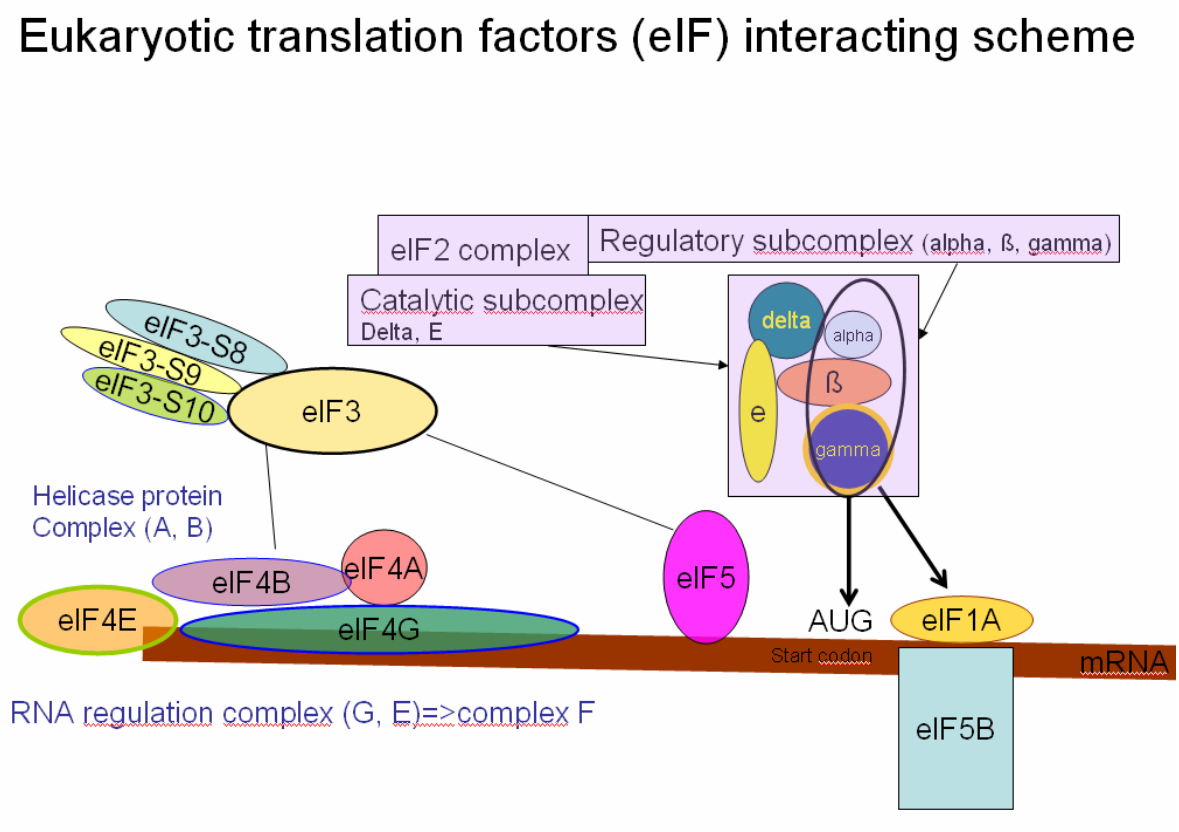


Figure 7: The graph shows the current experimental model of translational regulation by eukaryotic translation factors. All those above mentioned factors regulate gene expression on the level of mRNA translation.

TACC during cell division:

In the cell the main microtubule-organizing centers (MTOCs) are centromsomes (GLOVER, 1993; DESAI, 1997; KELLOGG, 1994). There are several proteins that are concentrated at centrosomes and have been shown to interact with microtubules (CULLEN, 1999; AVIDES, 1999; KELLOGG, 1989; KELLOGG, 1995; OEGEMA, 1997; KIDD, 1997; GARD, 1987). One of the proteins particularly intriguing is D-TACC that shows a strong interaction with Msps in Drosophila. Msps belongs to the XMAP215/ch-TOG family of MAPs kinases and can bind directly to microtubules and modulates their dynamics in terms of stabilization (GARD, 1987; VASQUEZ, 1994; TOURNEBIZE, 2000; SPITTLE, 2000; LEE, 2001).
Initially the transforming acidic coiled-coil (TACC) proteins were found as a group of proteins implicated in cancer (CHEN et al. 2000; SADEK, 2003; SILVERA, 2010; STILL, et al. 1999a; STILL, et al. 1999b). The unique feature of TACC is that at the ~200 amino acids at the carboxy terminus of TACC are predicted to form a coiled-coil hence the name TACC (LAPPIN, 2002). The physiological function of TACC has still to be elucidated but LEE *et al.* found out that D-TACC is essential for mitotic spindle function in the Drosophila embryo that is if D-TACC levels are reduced the centrosomes/spindle poles and the centrosomal microtubules appear destabilized (DO CARMO AVIDES, 1999). Furthermore when the function of D-TACC is perturbed by a mutation or antibody injection, centrosomal microtubules are shorter than normal. Embryos die owing to an accumulation of mitotic abnormalities (GERGELY, 2000). D-TACC interacts with Msps, the Drosophila homologue of XMAP215 in humans. This interaction influences the stability of centrosomal microtubules (GERGELY, 2000; BRITTLE, 2005; LEE, 2001). In Lee et al. it was shown that the TACC

domain does not directly interact with microtubules as the D-TACC domain did not show strong interaction with purified microtubules (PESET, 2008). Msps acts rather as a mediator to influence the stability of the D-TACC domain and microtubule binding site (LEE, 2001).

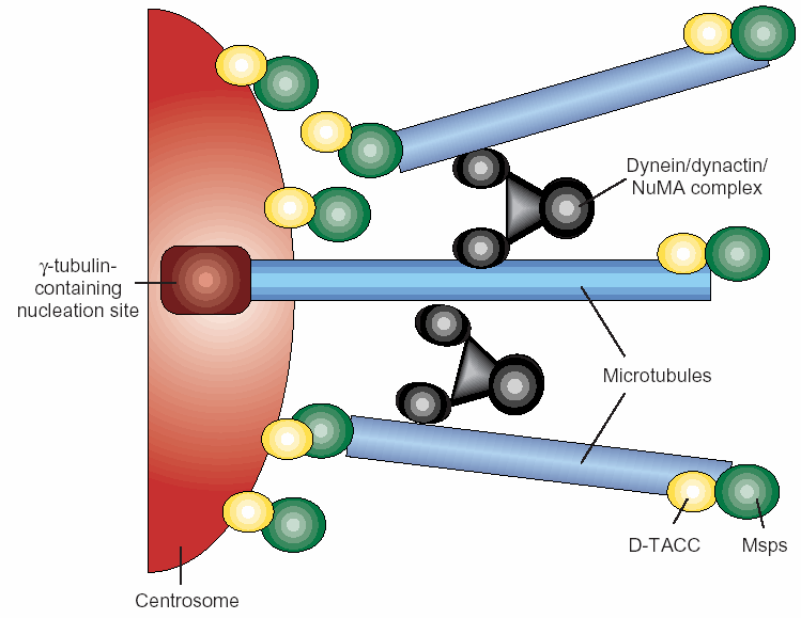


Figure 8: This image shows how centrosomal microtubules are stabilized by D-TACC and Msps. In the cell there is a concentration gradient of D-TACC and Msps molecules along the microtubules from the minus end to the plus end of the microtubule that is responsible for the microtubule stabilization. Along with other motor-protein complexes (e.g.: dynein/dynactin/NuMA) these molecules keep the microtubules focuses at the poles.

Picture from: LEE et al. (2001): *Msps/XMAP215 interacts with the centrosomal protein D-TACC to regulate microtubule behavior.*

2) Material and Methods:

Genetic strains and fly crosses:

Flies were cultured on a standard media. Crosses were carried out at 25 degrees Celsius. The following lines were used that have a genetic loss of function mutation and crossed them in a first step to GMR-GAL4 UAS CLASP flies. Orbit is the CLASP ortholog in Drosophila. To direct expression in the adult retina GMR-GAL4 was used. Flies were obtained form the Bloomington Drosophila Stock Center at Indiana University, Bloomington, IN, 47405-7005 USA. To identify homozygous embryos of the tacc1 allele the TM6B Ubx-lacZ balancer was used.

Genetic Screening:

A computer search on www.flybase.org showed different available mutants that have a mutation in one of the eukaryotic initiation factor (eIF) genes.

Name (Flybase)	Bloomington Center	FlyBase Genotype
eIF3-S10	15728	y1 w67c23; P{EPgy2}eIF3-S10EY04238/TM3, Sb1 Ser1
eIF3-S8	20433	y1 w67c23; P{wHy}eIF3-S8DG14606/SM6a
	16857	y1 w67c23; P{EPgy2}eIF3-S8EY07713/CyO
	20283	y1 w67c23; P{EPgy2}eIF3-S8EY11279/CyO
eIF3-S9	25337	w1118; Mi{ET1}eIF3-S9MB06914
	20931	y1 w67c23; P{EPgy2}eIF3-S9EY14430/CyO
eIF2-alpha	16584	y1 w67c23; P{EPgy2}eIF2B-αEY03991/TM3, Sb1 Ser1
eIF2-beta	10785	y1 w1118 PBac{PB}eIF2B-betac02002/FM7c
eIF2-gamma	10766	w1118; PBac{PB}eIF2B-gammac01931/CyO
	15441	y1 w67c23; P{EPgy2}eIF2B-δEY03558
eIF4A III	20978	w1118; P{GT1}eIF4AIIIGT-000230
	2781	eIF4AIII19 red1 e4/TM3, Sb1
	20914	y1 w67c23; P{EPgy2}eIF4AIIIEY14207/TM3, Sb1 Ser1
eIF4E-6	22438	y1 w67c23; P{EPgy2}eIF4E-6EY20966
eIF4E-7	25515	w1118 Mi{ET1}eIF4E-7MB06554a Mi{ET1}eIF4E-7MB06554b eIF4E-7MB06554
eIF1-A	23941	w1118; eIF-1A2232/TM6B, Tb1
	21496	y1 w67c23; P{wHy}eIF-1ADG10506
	16621	y1 w67c23; P{EPgy2}eIF-1AEY04874
	17203	w1118; P{EP}eIF-1AEP935/TM6B, Tb1
	23925	w1118; +/CyO; eIF-1A645/TM6B, Tb1
	11495	w1118; PBac{PB}eIF-1Ac04533/TM6B, Tb1
eIF5	22132	w1118 P{EPg}eIF5HP10800

Table 1: The following Bloomington flies that all have a mutation in a specific eukaryotic initiation factor gene were crossed to GMR-GAL4, UAS CLASP flies. The resulting progeny was then screened for differences in the eye phenotype.

Drosophila CNS Cultures and Immunocytochemistry:

Embryo collection:

20 to 30 flies from the tacc1/TM6B UBX lac Z genotype were placed in a collection chamber. These collection chambers consist of an egg lay cup 60 mm plastic Petri plates (Falcon Nr.: 1007) and a grape juice agar plate 333 ml grape juice, 2 g Methyl paraben (Sigma Nr.:H-5501), 33 g Dextrose and 30g agar in 1 liter of H_2O.

A small dab of yeast paste on the plate was attached to attract the females to lay eggs. The flies were prepared to lay eggs by feeding for a day with wet yeast paste (Fleischmann's Active Dry Yeast Nr.: 2133 and H_2O into a firm paste). Egg collectors were kept at 26°C for the entire time span. Embryos were harvested by washing them from collection plates with PBST loosening the embryos with a paintbrush. The embryos were transfered to one well of a multi-well plate with a disposable pipette.

Dechorionation and Fixation:

The embryonic eggshell the chorion was removed by incubating the plate in a 100 mm square Petri plates (Falcon Nr.: 1012) with 20 ml 50% Bleach (2.63% Sodium Hypochlorite) for 5-10 min.

A washing step was performed samples were rinsed extensively with PBST, then soaked in 20 ml fresh PBST in a 100 mm dish for 5min. Fixation was performed as follows embryos were incubated in a 100 mm dish with 10 ml n-heptane (Electron Microscopy Sciences Nr.: 54202535) plus 10 ml 4% formaldehyde (Fisher Scientific Nr.: 062032).

Incubation was performed on a shaker (VWR, Standard analog shaker) that

was set to 50-100 rpm for 25 min. For devitalization of the embryos, 5 ml of methanol (EM Science Nr.: 110608A) were added to the dish containing the embryos in heptane. After extensive rinsing with PBT buffer the embryos were labeled with an antibody. The primary antibody used was 1 D4 (anti-FasII) in a dilution 1:5 and it was incubated overnight at 4°C. After washing off the primary antibody the samples were incubated with goat anti-mouse-HRP serving as a secondary antibody that was heat inactivated at 65 °C for 30 min (Jackson ImmunoReseach, Nr.: 16210-072; 1:500). Detection of HRP was performed with the diaminobenzidine substrate peroxidase kit (Vector Laboratories). Samples were stored in 70% Glycerol/ PBS (Boehringer Mannheim Nr.: 100 649).

Microscopy and picture capture:

Images of all adult retinas were taken on a Zeiss Stemi SV6 dissecting microscope using a Spot Digital camera and Spot software (Diagnostics Instruments).

Image acquisition for the immunocytochemistry of the central nerve cord and peripheral motoneurons was obtained on a Nikon Eclipse 90i microscope using a Plan Apo 60x/1.40 oil Nikon objective equipped with a DXM1200C Nikon digital camera and NIS Elements software (Nikon, Japan). Figures were processed using Windows Picture and Fax Viewer (Microsoft, Windows XP).

3) Results:

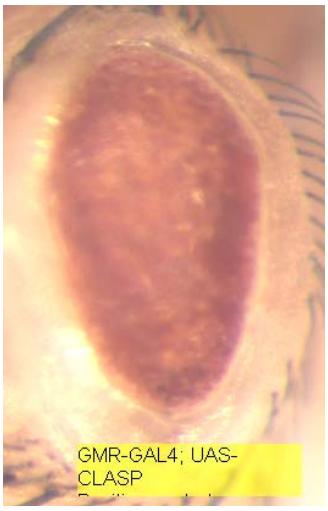
Genetic eye screen to study interactions between GMR-GAL4 UAS-CLASP and eukaryotic translation factors:

In this experiment I studied on the genetic network that has been elaborated on between CLASP and translation initiation factors which have been found in previous genetic as well as proteomic screens. The following genetic screen shows a selection of Drosophila flies that were ordered form the Bloomington Drosophila Stock Center at Indiana University. The below images show fly retina phenotypes were GMR-GAL4; UAS- CLASP was crossed to knock out mutant Drosophila with genes belonging to the eukaryotic translation initiation (eIF) group. The progeny of these flies was then visually inspected and multiple images series where taken and compared to GMR-GAL4; UAS- CLASP alone. Smaller eyes where defined as enhancers of the respective loci involved. Sometimes the size and shape of the retina also shows an abnormal surface that could be either rough or glossy. Suppressors on the other hand were defined as loci that result in larger eyes with a surface that is more patterned. The genetic interaction screen so far indicates no phenotypic variations.

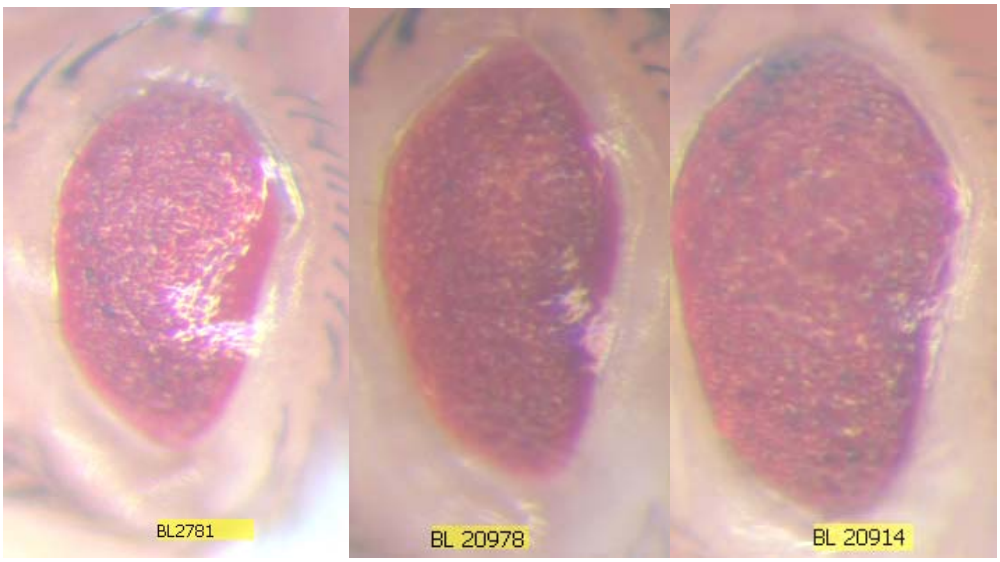
Nevertheless the recent literature points towards an interaction between translational regulation and CLASP. Msps the mini-spindles protein shows interaction with the plus- tip microtubule protein CLASP. The transforming acidic coiled-coil (TACC) that reportedly interacts with Msps, also interacts with CLASP. TACC has been shown to interact with CLASP and TACC is known to be under the influence of translation/RNA regulators (see Fig. 11).

A hypothesis was thus developed that eukaryotic initiation factors interact with CLASP.

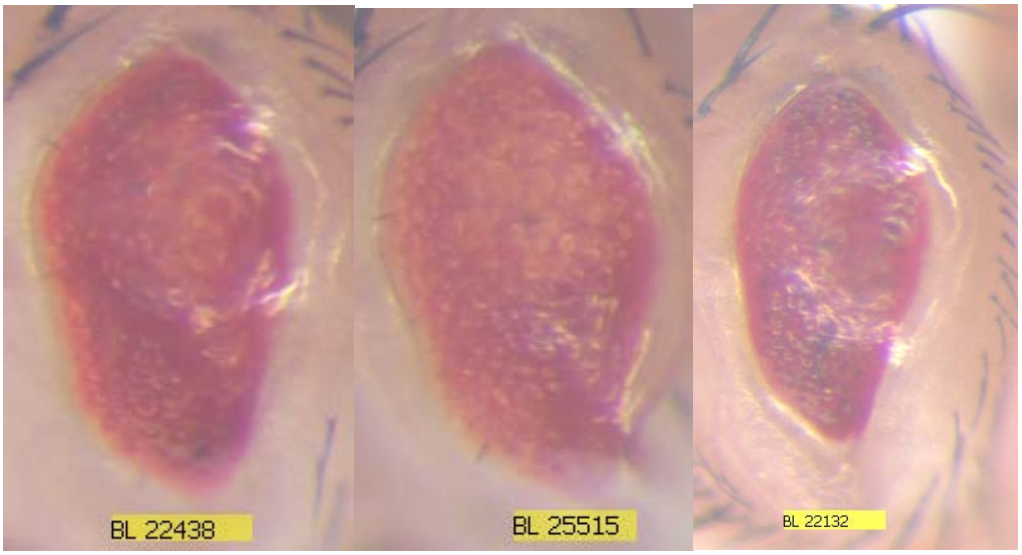
As depicted in the table below is a genetic eye screen in Drosophila:



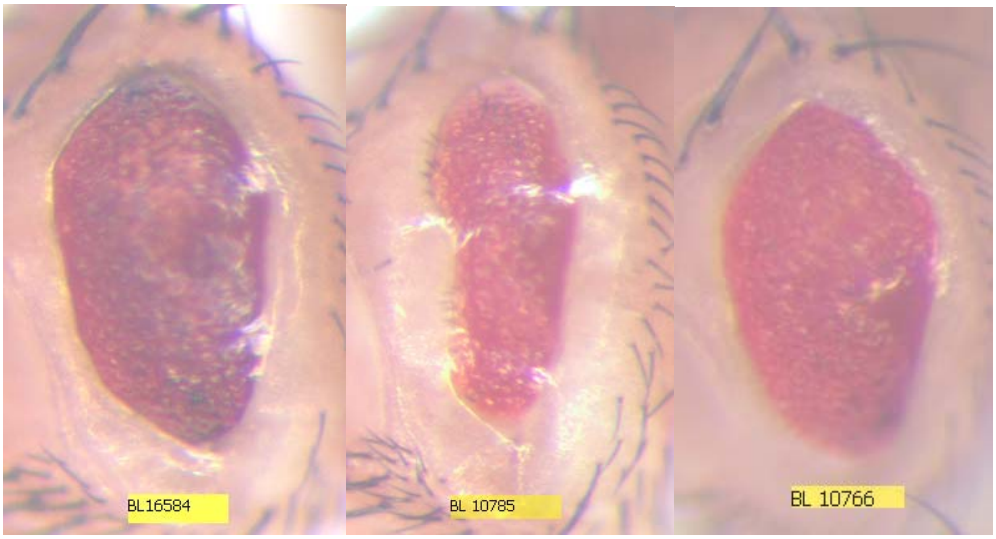
As a control I compared the GMR-GAL4; UAS-CLASP fly alone to the fly crosses in order to determine any change in the eye. The GMR-GAL4; UAS-CLASP flies alone were bred and compared to flies that were crossed between GMR-GAL4; UAS-CLASP and various eukaryotic initiation factors (eIF). In the genetic screen so far the phenotypic eye images show no real difference in regard to the hypothesized CLASP and eIF interaction.



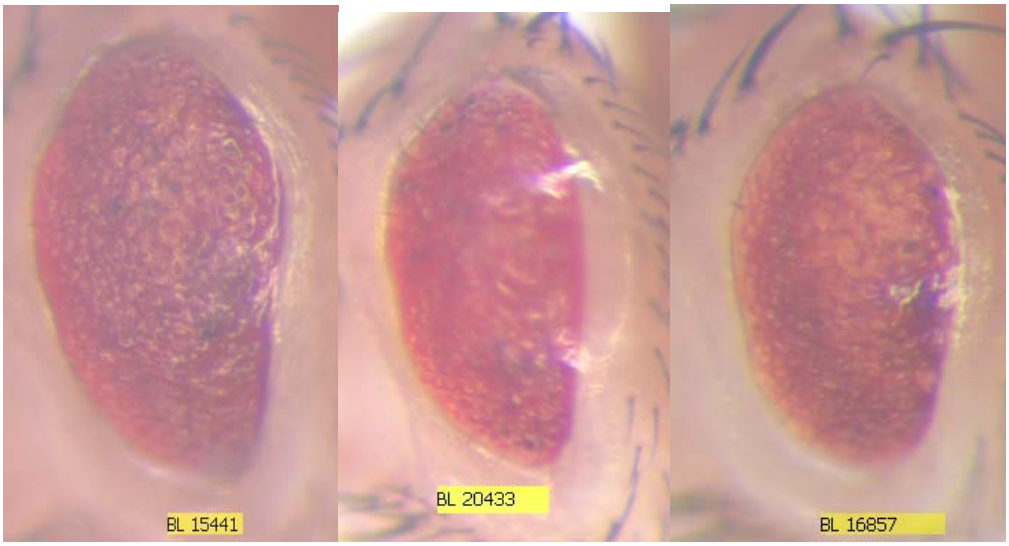
The above flies are knock- outs for the gene eIF4A III. They were crossed to GMR-GAL4; UAS- CLASP. The numbers in the picture show the Bloomington stock number and the full fly genotype can be found via the homepage of the Bloomington fly stock center (http://flystocks.bio.indiana.edu).



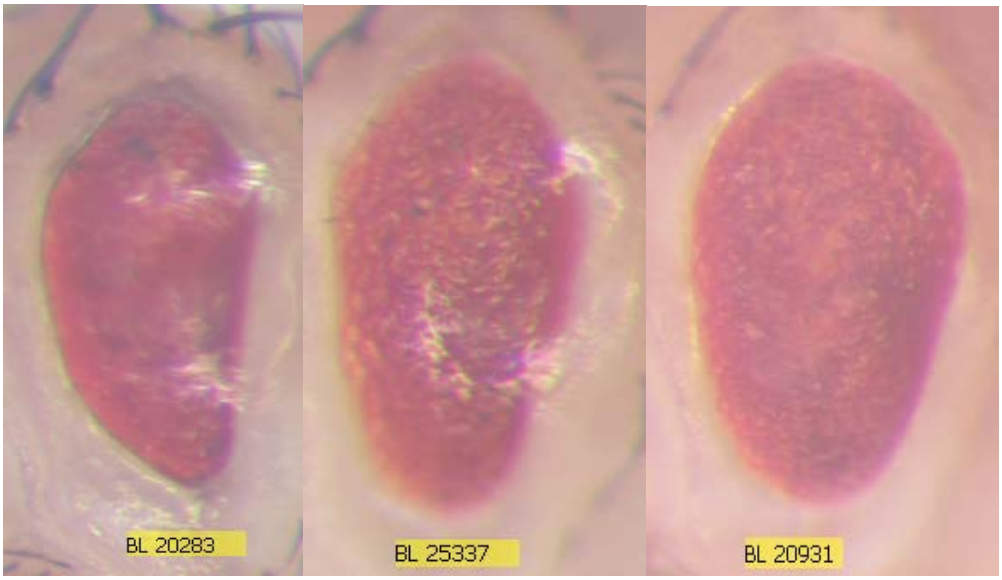
The following fly eyes BL 22438 and BL 25515 depict Drosophila eyes that have a knock out in the eIF4E gene. The shape as well as the roughness of the eye shows no significant difference when compared to the GMR-GAL4; UAS- CLASP offspring.



Bloomington fly stock numbers BL 16584 disrupts the eIF2-alpha gene. The BL10785 fly has a knock out in the gene called eIF2- beta. Fly number BL 10766 has a knock out in the eIF2-gamma gene and stock number BL 15441 knocks out the gene eIF2-delta. BL10785 turned out to be a false positive because the progeny appears to have a smaller eye phenotype but looking at the eIF2- beta fly stock it turned out that the phenotype by itself appears to have this retina phenotype.



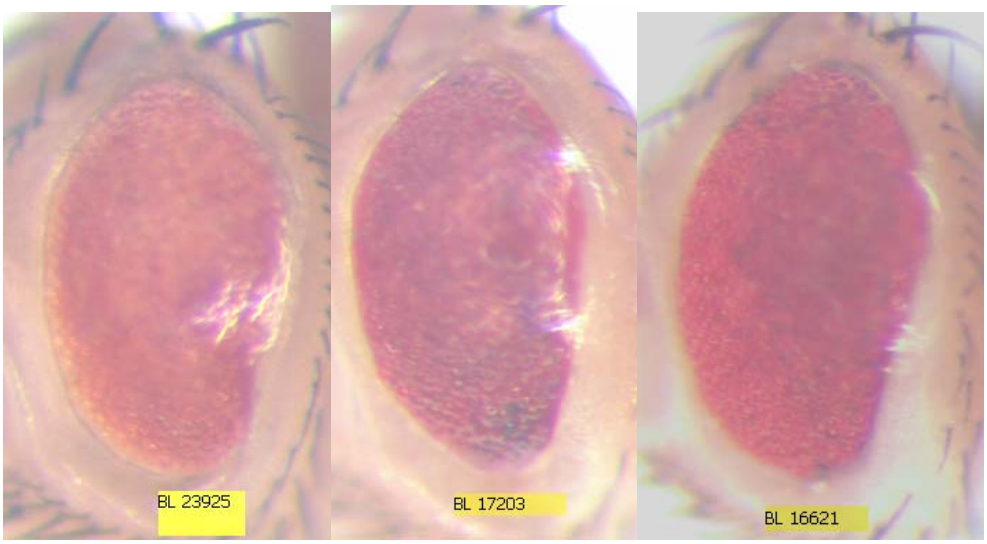
The stock number BL15441 has a genotype where eIF2-gamma has been knocked out. BL20433, BL16857 as well as BL20283 have knock- outs for gene eIF3-S8.

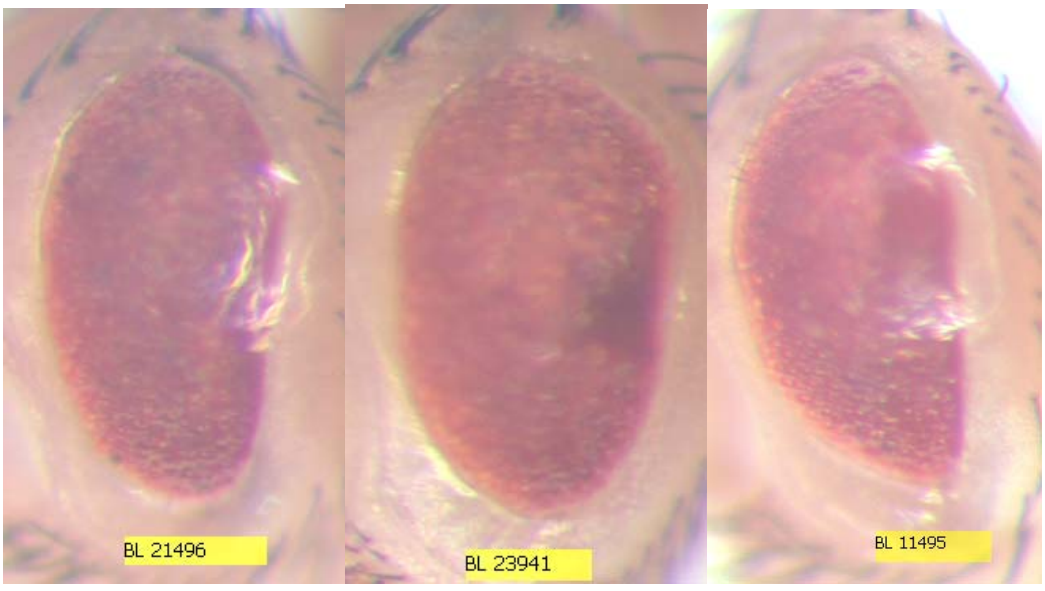


In BL25337 and BL20931 the gene eIF3-S10 is knocked-out.

Drosophila eye screen in mutant genotype for the gene eIF1-A:

The six images below show fly retina phenotypes were were crossed to knock out mutant Drosophila with genes that knock out the eIF1-A gene. The progeny of these flies shows no remarkable phenotypic difference. Bloomington stock number BL11495, BL 16621, BL 17203, BL21496, BL 23925 and BL 23941 were ordered and crossed to GMR-GAL4; UAS-CLASP. The images show the retina of the progeny.





The phenotype of mutant tacc1 deficiency fly embryos:

The Drosophila retina can be regarded as a suitable method to tackle questions that elaborate on genetic interaction between CLASP and members of the eIF family. In contrast, dissecting Drosophila embryos and examining the embryonic nervous system provides a system to detect abnormalities in mutant fly embryos during their development. Axon fascicles that are restricted to either side of the midline by Slit and its Robo receptor signalling can be visualized at stage 17 with anti-Fasciclin II (DICKSON, 2006; JAY, 1999).

My goal in this experiment was to study if the Drosophila embryo, that has a loss of function for TACC, shows axon guidance defects during its development at the stage 17. At this stage the Drosophila embryo has completed its ventral nerve cord development. The characteristic feature after adequate staining with 3,3'-Diaminobenzidine (*DAB*) shows in the wildtype a characteristic three line fascicle formation on either side of the midline (see Figure 9). The faint but exact vertical midline crossing in the image below indicates a developmental stage that might be judged as stage 16 and a half.

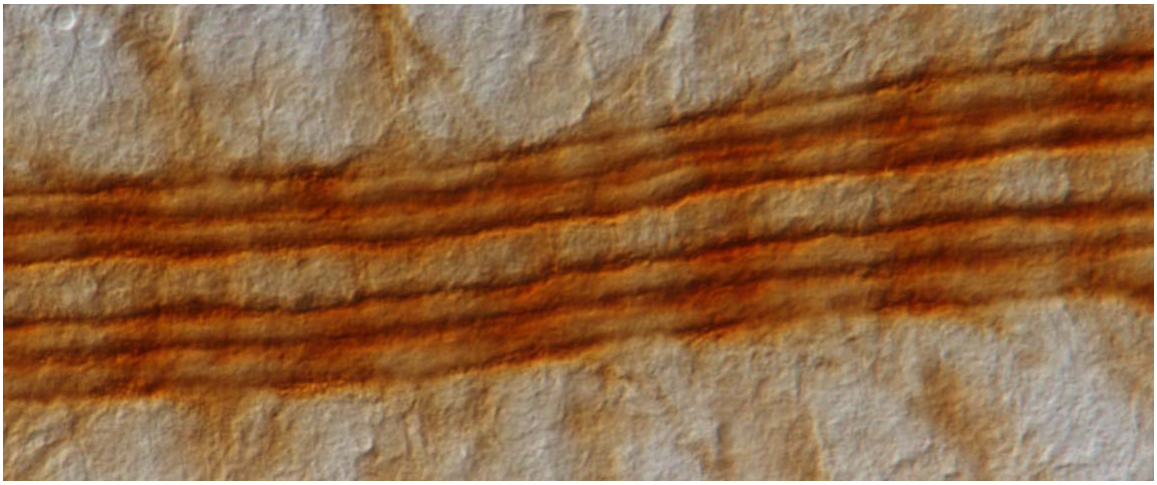


Figure 9: This image shows the central nervous system (CNS) of a Drosophila melanogaster at the stage 17. Staining was performed using a diaminobenzidine substrate peroxidase kit. Several CNS segments in a wild-type embryo are shown stained with mAb 1D4 at stage 17, revealing three large parallel fascicles of longitudinal axons on each side of the midline. There are three fascicles lateral to the midline. In every segment at this developmental stage one can see vertical fascicle crossing of the midline. This midline crossing disappears at a later stage. Only mutant with neuro-specific developmental phenotypes show a distinct fascicle midline crossing.

TACC1 loss of function (LOF) mutant shows axon guidance defects:

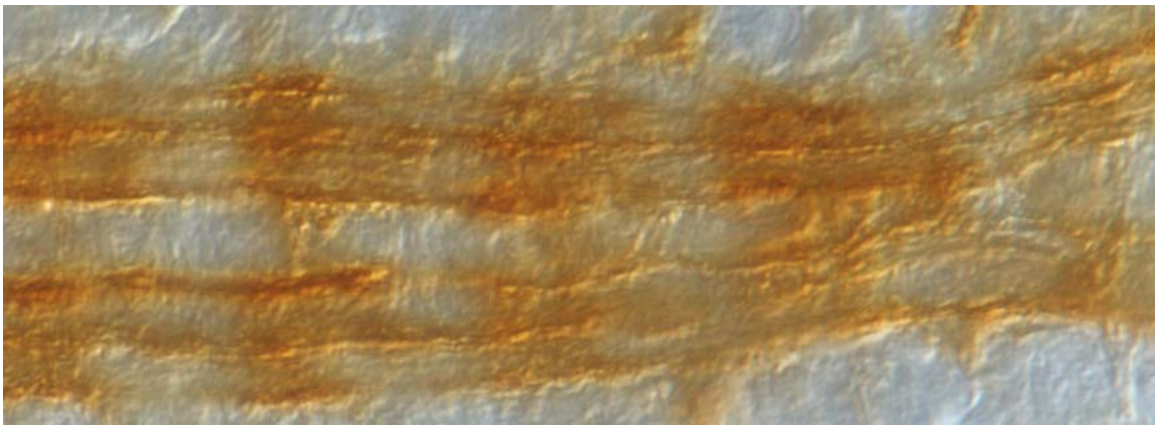


Figure 10: This image shows a stage 17 drosophila embryo ventral nerve cord that was dissected and stained in monoclonal 1 D4 (anti-FasII) antibody. Although three fascicles can be seen in each hemisegment, they are often disorganized, often fusing together or extending in abnormal directions.

4) Discussion:

The cytoskeletal function and regulation by specific regulatory proteins have been studied extensively over the past two decades (SANCHEZ et al. 2007). It allows us to better understand the genetic interaction of how guidance cues are transduced to direct cytoskeletal re-organisation. Abnormalities in axon guidance have been associated with multiple hereditary neurological disorders and thus research work may let us understand better how axon guidance works and defects develop, which genes are involved and possibly how to prevent or alleviate developmental malformations and defects.
In order to elucidate genetic regulatory mechanisms forward genetic screens with Drosophila melanogaster as a model organism are an adequate tool to address such questions (JOHNSTON, 2002). The screen with the Bloomington knock out flies for eukaryotic initiation factors showed no obvious phenotypic change. But it is discussed that Msps that is known to be a common partner of TACC and the CLASP-Abl pathway, not only interacts with CLASP and with Abl but also shows a connection to translational regulation factors complementing a broader interactome picture of CLASP. The genetic interaction screen so far indicates no phenotypic variations that would point towards a genetic link between CLASP and translation/RNA regulation. However, this data suggests further investigation.

As in the recent literature there is compelling evidence that highlights Msps a mini-spindles protein that shows interaction with the plus- tip microtubule protein CLASP. On top of that the transforming acidic coiled-coil (TACC) that reportedly interacts with Msps and TACC, also interacts with CLASP. TACC has been shown to interact with both CLASP and TACC. All this dada points towards a regulation of CLASP by translation/RNA regulators (see Fig. 11).

Building on this dada and using CLASP and the previous data of CLASP interactors (LOWERY et al., 2010) this screen elaborated on the hypothesis that translational regulation could also be involved in the overall process of microtubule-actin cross talk. CLASP has not previously been connected with translation/RNA regulation, but recently it has been shown that there are two genes namely CG31957 and tra2 a gene that is implicated in mRNA regulation and splicing that possibly links translational regulation with the cross-talk between cytoskeletal regulation (LOWERY et al., 2010).

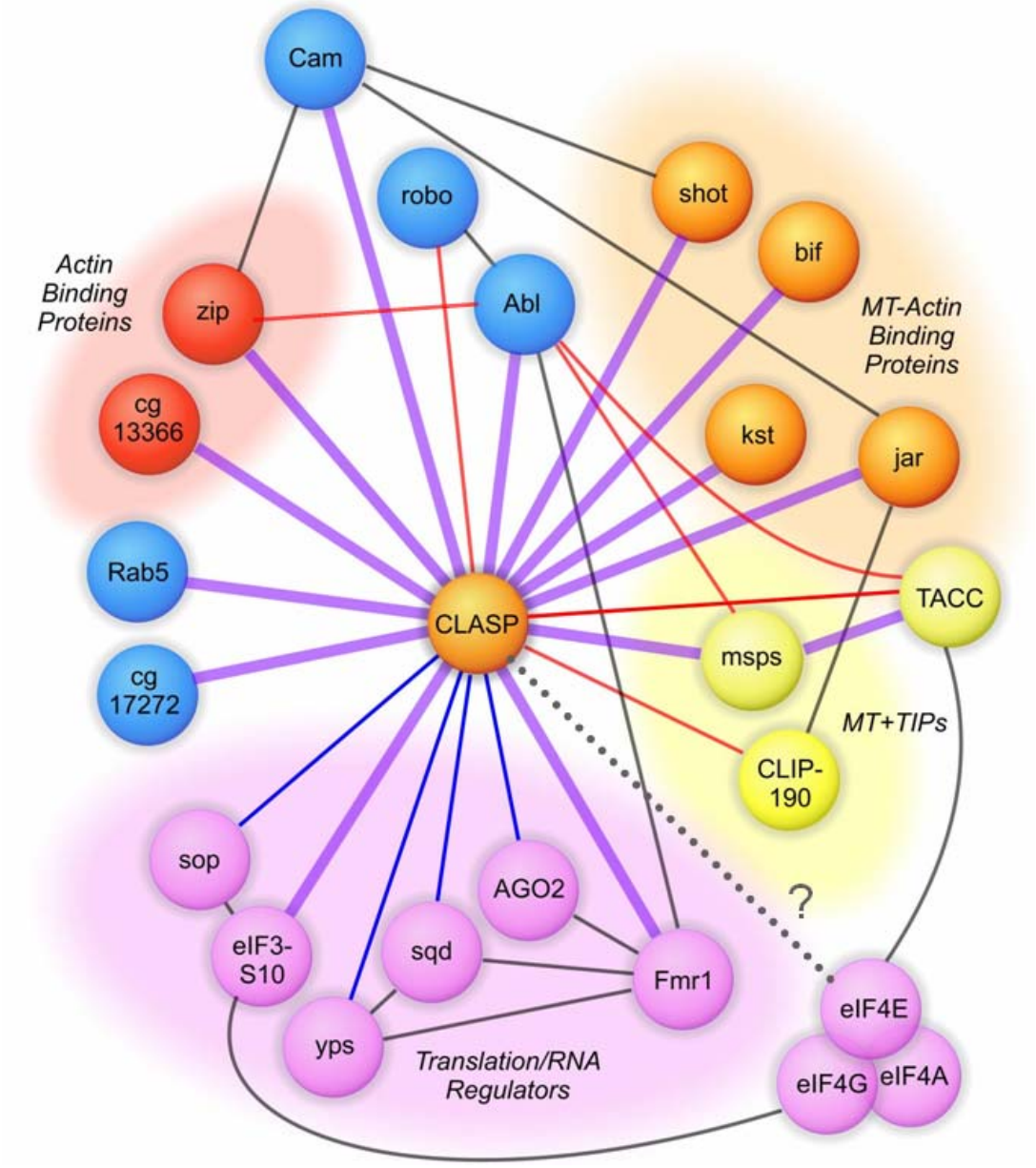


Figure 11: The CLASP interactome. This figure shows the current working model of Professor Van Vactor lab elaborating on CLASP interactors.
Thick purple lines indicate interaction supported by both genetic and proteomic data. Blue lines represent physical interaction based on proteomic data, whereas red lines represent genetic interaction. Black lines denote previously known interactions. Blue nodes are components of the receptors/signaling category, pink nodes are mRNA and translation regulators, whereas cytoskeletal components are split into three groups: red are actin-binding proteins, orange are MT-actin binding proteins, and yellow are MT+TIPs.

Graph was modified in courtesy of Dr. Laura Anne LOWERY.

Conclusion and perspectives:

This work was done to give some insight into cell interaction pathways that advance growth cones during central nerve cord development. The combination of genetics, proteomics, and biomedical techniques as well as other technological advances, showed several factors that all need careful investigation. The genetic regulation of microtubule plus tips by eukaryotic initiation factors is a surprising finding and therefore further study can be kept up to draw a complete picture of the CLASP interactome.

Furthermore, mechanisms involved in axon guidance are thought to influence the ability of axons to regenerate after neural injury and so this basic research may contribute to be able to use this information to design treatments to allow regeneration in the future (NICLOU, 2006). There is additionally a growth inhibitory nature of injured adult central nervous system that is to blame on a mixture of various repellent factors. A major quest for neuroscientist is to reestablish innervation lost as a consequence of injury and thus make proximal and distal neuronal ends located close to the injury site grow together again (GIGER, 2010).

In addition to that, the signaling proteins studied here show abnormal regulation in certain cancers (CHEN, H. M. et al., 2000; STILL, I. H. et al., 1999). Thus, the research carried out here and methods used are of broad biomedical significance.

It is necessary to expand the study of factors such as the eIF RNA regulation/translation factors, both at the sub-cellular level and in combination with genetic strategies such as the phenotypic eye screen in *Drosophila* to gain further insight into how nerve tissue development and how diseases may arise. It allows us to better understand how guidance cues are transduced to direct cytoskeletal re-organisation. From this fundament we can then learn to develop strategies to cure diseases and disorders.

5) Summary:

The goal of this work was to study relevant functional partners in the network of CLASP, a protein factor that is involved in microtubule and actin networks. Among these modifiers of CLASP is a group of translation/RNA regulators.

In a genetic and a proteomic screen translation/RNA regulators belonging to the eukaryotic initiation factor (eIF) groups pulled down. This thesis elaborates on a genetic interaction between the eIF group and Orbit/CLASP. A genetic screen where GMR-GAL4; UAS- CLASP flies were crossed to various knock out Drosophila flies, so far revealed no compelling evidence that the resulting progeny showed any difference in their eye phenotype. However when investigating the phenotype of tacc1 allele mutants that are deficient for the tacc1 allele indicate that a genetic interaction between CLASP and RNA/translational factors is highly suspected.

6) Zusammenfassung:

Einer der grundlegendsten Prozesse in der Zellbiologie ist die Zellmotilitat. Das Verstaendniss wie sich zum Beispiel Neuronen fortbewegen anhand der Muster von Aktin und Mikrotubulus ist elementar im Verstaendniss von Neuronalem Axon Wachstum in der Entwicklung von Organismen. Diese Arbeit beschaeftigt sich mit der Entwicklung von Nervenzell- Strukturen bei Fruchtfliegen als Modelorganismus; und ein zentraler Faktor CLASP genannt, zeigt Interaktionen mit einer Gruppe von translationallen Regulations Faktoren die weitere Untersuchung benoetigen. Diese Erkenntnisse aus genetischen sowie protein- studierenden Untersuchungen haben dann zu der Aufstellung der Hypothese gefuehrt die die Grundlage dieser Arbeit wurde. Die da lautet, dass translationale Kontrolle beteiligt ist in plus- tip Funktion waehrend der Fuehrung von Axon im Nervensystem bei Drosophila melanogaster. Es wurde ein genetischer Retina Screen studiert um die genetische Interaktion von TACC1, einem Faktor der wissentlich mit CLASP und einer Gruppe von translationallen Regulations Faktoren zusammenspielt. Auch wurden biochemische Faerbungen durchgefuehrt die Axon Missbildungen im Zentralem Nervensystem der Fliege zeigen, bei genau jenen Phenotypen die eine Mutation in TACC1 Allel haben. Die Ergebnisse sind vielversprechend und brauchen daher weitere Untersuchungen.

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