

Regulation and function of *Pax-6* during
head and eye development in
Drosophila melanogaster

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Umeå 2012

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ISBN: 978-91-7459-348-8

ISSN: 0346-6612

Cover Photo by: Sandra Viklund (www.hojnadesign.se)

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Elektronisk version tillgänglig på <http://umu.diva-portal.org/>

Printed by: Print o Media, Umeå, Sverige 2012

Till min familj

“The breaking of a wave cannot explain the whole sea”

/ Vladimir Nabokov (1899-1977)

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ABSTRACT

In *Drosophila melanogaster*, *eyeless* and *twin of eyeless* have important function during eye development. Mutants of both genes give a variety of eye and head phenotypes with the strongest being almost headless meaning that they lack all structures derived from the eye-antennal disc. *toy* the first eye specification gene expressed in the regulatory network that leads to eye formation and the gene activates *eyeless*. What regulates *toy* is still not clear and has been the focus of this thesis. In Paper I, we analysed part of the upstream region of the *toy* gene to be able to drive reporter genes with the expected expression pattern in embryo and larval tissues. We found that a 1300-bp region surrounding the *toy* transcription start is important for correct Toy expression during embryonic and larval development. We also tested for possible redundancy between the *toy* and *ey* genes by rescue experiments on some lethal alleles in both genes and found that Pax-6 proteins can substitute for each other concerning both head structures and survival. However, rescue is only partial, indicating that the proteins are not fully compatible or that the levels of expression are not sufficiently reproduced by the artificial Gal4-UAS system. Furthermore, we show that inhibition of apoptosis increased survival in strong *toy* mutants, but did not improve eye phenotypes. In Paper II, we searched for possibly upstream regulators of *toy* and found that the head gap gene *empty spiracles* changed the expression pattern of Toy significantly in the embryonic eye-antennal primordium. By clonal analysis and ectopic expressions, we made the conclusion that Ems acts as a repressor of *toy* during late embryonic development and also at later developmental stages. In Paper III, we investigate presumptive *toy* enhancer regions within the intron sequences of the gene. Generation and examination of transgenic lines showed that there might be an enhancer region driving *toy* expression in the embryonic ventral nerve cord within intron 2.

This thesis is based on the following article and manuscripts referred to in the text by their roman numerals.

Paper I

Jacobsson L^{*}, Kronhamn J^{*} and Rasmuson-Lestander A (2009). The *Drosophila Pax6* paralogs have different functions in head development but can partially substitute for each other, *Mol. Genet Genomics*, May; 30 (282): 217-231 ^{*}Authors have contributed equally.

Paper II

Jacobsson L^{*}, Skottheim Honn J^{*}, Ekström K and Rasmuson-Lestander Å (2011). Empty spiracles is repressing *twin-of-eyeless* in the *Drosophila* embryonic head, *Submitted under revision* ^{*}Authors have contributed equally.

Paper III

Jacobsson L and Rasmuson-Lestander Å (2011). Identification of regulatory regions within the intron sequence of the *toy* gene, *Manuscript*

ABBREVIATIONS

Genes and proteins are written differently. All genes are in italics and proteins are not, for example *toy*, (gene) and Toy (protein).

<i>btd</i>	<i>buttonhead</i>
<i>dac</i>	<i>dachshund</i>
<i>dpp</i>	<i>Decapentaplegic</i>
<i>ems</i>	<i>empty spiracle</i>
<i>en</i>	<i>engrailed</i>
<i>ey</i>	<i>eyeless</i>
<i>eya</i>	<i>eyes absent</i>
<i>eyg</i>	<i>eyegone</i>
HD	Homeodomain
<i>hh</i>	<i>hedgehog</i>
<i>hth</i>	<i>homothorax</i>
MB	muschroom bodies
MF	Morphogenetic Furrow
<i>N</i>	<i>Notch</i>
<i>otd</i>	<i>orthodenticle</i>
PD	Paired domain
<i>so</i>	<i>sine oculis</i>
<i>sey</i>	<i>small eye</i>
<i>toy</i>	<i>twin of eyeless</i>
<i>toe</i>	<i>twin of eyegone</i>
VNC	Ventral nerve cord
<i>wg</i>	<i>wingless</i>

SUMMARY IN SWEDISH

Bananflugan, eller *Drosophila melanogaster* som den heter på latin, har förekommit i forsknings-sammanhang i lite mer än 100 år. Dess popularitet inom forskning beror bland annat på att de har en liten arvs massa med bara ca. 14000 gener och att de flesta av dessa gener har ett gemensamt ursprung med gener hos andra arter, även människan. Med tiden har det även utvecklats många tekniker som gör att bananflugan idag är den mest lätthanterliga multicellulära organismen för genetisk analys.

Livscykeln hos bananflugorna börjar med ett embryo som sedan utvecklas till en larv. Larven har tre stadier, första, andra och tredje larvstadiet innan den förpuppas. I puppan genomgår larven metamorfos och bildar den vuxna flugan. Under embryostadiet är segmentering en viktig process. De flesta delar hos den vuxna insekten utvecklas från en serie av segment i embyot. Kroppen och huvudet segmenteras genom två olika processer och det styrs med hjälp av olika gener. Tre gener som är viktiga för huvudet och hjärnans utveckling är; *orthodenticle (otd)*, *empty spiracles (ems)* och *buttonhead (btd)*. Dessa gener tillhör familjen gap-gener och mutationer av dessa leder till ett mellanrum (gap), i den normala kroppsplanen.

En annan grupp av gener som är viktiga under utvecklingen av bl.a. njurarna, bukspottkörteln, nervsystemet och ögonen är Pax- generna. Hos ryggradsdjuret finns det nio Pax gener benämnda från Pax 1-9. *Pax-6* har fått mycket uppmärksamhet på grund av dess roll i ögonutvecklingen. Genen isolerades först hos människor, mus och zebrafiskar men har också hittats i många andra djurarter, som t.ex. bananflugan. Att den var funktionellt viktig upptäcktes genom studier av den mänskliga ögonsjukdomen aniridia och dess homolog hos möss kallad *small eye (sey)*. Aniridiapatienter har underutvecklad iris och nedsatt detaljseende. *sey/+* möss har som namnet antyder mindre ögon. Homozygota mutationer av *Pax-6* hos människor leder till avsaknad av ett eller båda ögonen, defekter på det centrala nervsystemet och man överlever inte. Hos möss så saknar embryona

båda ögonen näsan och har svåra hjärnskador och är även här förknippat med dödlig utgång.

Identifieringen av genen *eyeless (ey)* som en *Pax-6* homolog hos bananflugan visar att den även har en viktig funktion i ögon utvecklingen hos insekter. Hos bananflugorna finns även en till *Pax-6* gene, *twin of eyeless (toy)*. Båda dessa gener har mer än 90% likhet med ryggradsdjurens *Pax-6* gen.

Bananflugans synsystem består av två fasettögon samt tre enkla ögon på toppen av huvudet, som kallas ocelli. Båda stukturerna utvecklas från en grupp av celler i embryonalstadiet. Både *toy* och *ey* har viktiga funktioner i utvecklingen av bananflugans ögon, då avsaknad av någon av generna leder till flugor som har mindre eller helt saknar ögon och i värsta fall även saknar huvud.

Den här avhandlingen bygger på studier av reglering hos *toy* genen och hur de två *Pax-6* generna samverkar med varandra under utvecklingen av ögon.

Vi har funnit att *Toy* och *Ey* har specifika uppgifter när det gäller ögonutvecklingen hos bananflugorna. *Toy* verkar vara viktigare för utvecklandet av ocelli och *ey* för fasettögonen. När det gäller överlevnad så kan dock generna ersätta varandra. Vidare har vi även funnit att *gap-* genen *ems* har en hämmande roll på *Toy* under utvecklandet av ögon. Om denna funktion är direkt eller indirekt är ännu oklart. Slutligen har vi även hittat en möjlig reglerings region i intron 2 av den genomiska sekvensen av *toy*. Potentiella hotspots med olika transkriptionsfaktorer har bekräftats men det är inte utrett om de har någon faktisk roll i regleringen av *toy*.

INTRODUCTION

The fruit fly - *Drosophila melanogaster*

History

To many people this little fly is just an insect suddenly appearing around your fruit bowl and therefore you know it as the fruit fly. For others it plays a more significant role. For more than a 100 years, this little creature has been involved in various research studies, starting with the hereditary studies of Tomas H. Morgan and the discovery of the first sex linked mutant allele, *white*. Now there are thousands of scientists working on many different aspects of the fruit fly, helping us to deepen our understanding of developmental biology (Rubin and Lewis, 2000).

Why flies?

The fruit fly has many advantages when it comes to science. They are small, easy to work with and cheap to cultivate in a laboratory. They have a small sized genome with approximately 14000 genes, and most genes have homologs in other species including humans. About 75% of known human disease genes have a recognizable match in the genome of fruit flies. For a long time, scientists have been developing a wide range of techniques that make *Drosophila* one of the most easily handled multicellular organisms for genetic analysis.

Development

Life cycle

The lifecycle of *Drosophila melanogaster* is short compared to many other multicellular model organisms. It takes about 10 days at 25 degrees to go from fertilization to the hatching of a new adult fly. During these days several different stages of development take place

beginning with the embryo, that after 24 hours develops into a first instar larva. The larva molts two times into the second and third instar before it forms a pupa. In the pupa case, the larva undergoes metamorphosis and forms the adult fly (Figure 1).

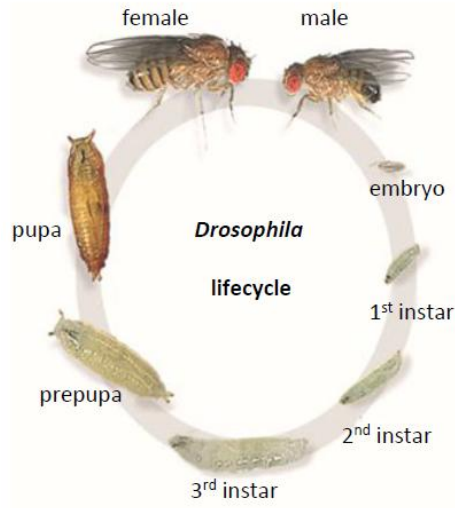


Figure 1. The lifecycle of *Drosophila melanogaster*
Modified and reprinted with permission from Flymode.

Embryogenesis

In insects, segmentation is a fundamental aspect of embryonic development. Almost all parts of the adult insect have derived from a series of segments.

In *Drosophila*, four classes of maternal gene products establish the coordinates of the embryo. The terminal, anterior, posterior and dorsal/ventral groups of genes, encode these gene products (Finkelstein and Perrimon, 1991). Pattern formations in the trunk (thorax and abdomen) are done in a hierarchical way, starting with maternal information from the anterior and posterior groups, which then activates the zygotic gap genes. The gap gene products divide the embryo into broad domains and activate the pair-rule genes that

establish the early parasegments. To finalize the metameric pattern of the embryo, the pair-rule proteins initiate the expression of segment polarity genes, resulting in the formation of fourteen segments (Figure 2). The segments are specified by the homeotic selector genes of the Antennapedia complex and Bithorax complex (Finkelstein and Perrimon, 1991; Ingham, 1988).

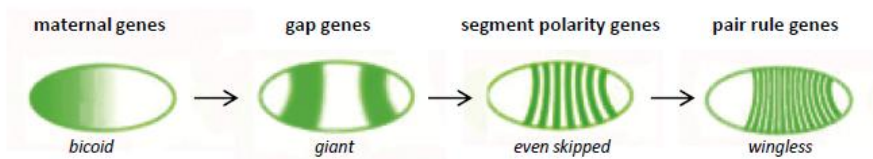


Figure 2. Segmentation hierarchy during embryogenesis in *Drosophila*

The fly embryo is subdivided into the head and the trunk, (thorax and abdomen) through two different mechanisms (Cohen and Jürgens, 1991; Finkelstein and Perrimon, 1991).

Embryonic head development

The head of the embryo is divided into two distinct domains, the anterior terminal domain and the segmented region. The non-segmented acron and the labral segment make up the terminal domain and require input from the terminal group of maternal genes such as *torso* and *nanos*. The segmented region consists of six segments; ocular, antennal, intercalary, mandibular, maxillary and labial. These segments need maternal contribution from the anterior group of genes, mainly *bicoid* (Grossniklaus et al., 1994).

Three posterior head segments the mandibular, maxillary and labial, also called the gnathal segments, are subdivided in the same hierarchical manner as the trunk segments. Patterning of the anterior head segments is done by a different mechanism with no contribution of pair-rule and HOX-cluster genes. Instead, these segments require the overlapping expression of head gap genes. Mutational analysis of the head gap genes *orthodenticle (otd)*, *empty spiracles (ems)* and *buttonhead (btd)* have shown that these genes

also function as homeotic selector genes specifying segmental identity in the head (Cohen and Jürgens, 1990; Schmidt-Ott et al., 1994).

During embryogenesis, the segments of the head will undergo some complex morphogenetic movements, called head involution that will shape the head of the larvae. In the larva, three major discs are formed, the eye-antennal disc, the labial disc and the clypeo-labral disc, that during metamorphosis will give rise to the adult head (Younossi-Hartenstein et al., 1993).

Gap genes

During development, several groups of genes play major roles in organogenesis and patterning. In the next sections, two of these groups will be discussed further, the *Gap* genes and the *Pax* genes, as well as some of the members of these gene families.

Gap genes encode transcription factors involved in early patterning of the embryo. Mutations of such genes usually lead to a “gap” in the normal body plan such as deletions in cuticular structures, the absence of sensory organs and the loss of segmentation markers, as *engrailed (en)* and *wingless (wg)* (Grossniklaus et al., 1994; Jym, 1995; Schmidt-Ott et al., 1994). The head gap genes are expressed in overlapping broad anterior stripes at the blastoderm stage. Later these stripes will narrow into smaller patches in the ectoderm of the head and some of them correspond to different parts of the brain (Younossi-Hartenstein et al., 1997).

empty spiracles

empty spiracles (ems) encodes a homeodomain containing transcription factor that is required during embryogenesis for head and brain development, specifically the development of the antennal head segment (Lichtneckert and Reichert, 2005).

Ems loss-of-function mutations result in a gap-like phenotype in the embryonic head and brain. This includes deletions in cuticular structures, the loss of several cephalic sensory structures and the absence of *engrailed (en)* and *wingless (wg)* expression from the intercalary, antennal and preantennal segments (Cohen and Jürgens, 1990; Dalton et al., 1989; Jym, 1995; Schmidt-Ott et al., 1994; Walldorf and Gehring, 1992; Younossi-Hartenstein et al., 1997).

During embryo development, *ems* is expressed in two independently regulated patterns, one in the head region and the other in the ventral nerve cord. The *ems* head-specific expression pattern is seen prior to cellular blastoderm stage in a single circumferential stripe at the anterior end of the embryo and continues until early germ-band extension. Later, *ems* is seen in a metameric expression pattern in ectodermal and neural cell patches in all trunk segments (Dalton et al., 1989; Walldorf and Gehring, 1992).

During postembryonic brain development, *ems* is expressed in two neuroblast lineages of the deutocerebral brain that give rise to the antennal lobe projection neurons and local interneurons; *ems* function is necessary for the specification of these olfactory interneurons, as well as for targeting of their neurites in the antennal lobe (Lichtneckert 2008).

orthodenticle

Pattern formation in the head vertex requires the homeodomain protein Orthodenticle (*Otd*). During second instar larvae, *otd* is ubiquitously expressed in the eye antennal disc but is gradually restricted to the vertex primordium at early third instar larval stage (Royet and Finkelstein, 1995, 1997). The restricted expression of *otd* initially depends on *Wg* and Hedgehog (*Hh*) signaling. Later on these signals become redundant and a positive autoregulatory loop, by *otd* itself, sets in to maintain *otd* expression during the last stages of vertex primordium development (Blanco et al., 2009; Royet and Finkelstein, 1996).

buttonhead

While *ems* and *otd* encode homeodomain-containing proteins *btd* encodes a zinc-finger protein that regulates transcription of segment polarity genes *wingless* and *engrailed* (Jym, 1995; Wimmer et al., 1993). Initial expression of *btd* is like *otd* and *ems*, found in a stripe domain in the blastoderm embryo. This domain covers the anlagen of the antennal, intercalary and mandibular segments, which fail to develop in *btd* mutant embryos (Wimmer et al., 1993). Activation of blastoderm *btd* expression is as for both *ems* and *otd* dependent on the anterior morphogen *bicoid* (Dalton et al., 1989; Gao and Finkelstein, 1998; Hartmann et al., 2001; Walldorf and Gehring, 1992). The posterior and dorsoventral morphogenes, *hunchback* (*hb*) and *dorsal* (*dl*) are required for the correct spatial expression of *btd* (Wimmer et al., 1995).

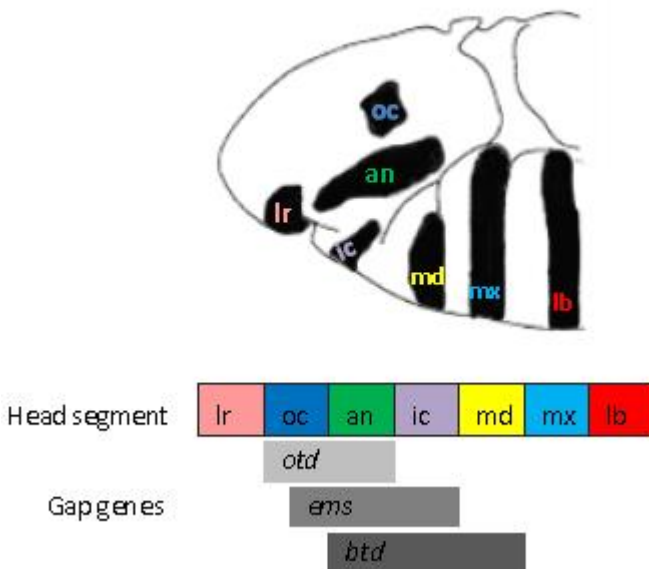


Figure 3. Head segments and gap gene expression pattern

Head segments are color coded, each color represents one segment in the head of the embryo. Expression and overlap between head-gap genes in different head segments are shown below in grey bars. (lr) labral, (oc) ocular, (an) antennal, (ic) intercalary, (md) mandibular, (mx) maxillary, (lb) labial.

Pax genes in vertebrates and *Drosophila*

Pax proteins are divided into five subgroups comprising *Drosophila* and vertebrate genes based on genomic structure, sequence similarity and conserved protein function. In mammals, there are nine Pax genes, named Pax 1-9 and several of them have homologs in *Drosophila*. (Breitling and Gerber, 2000; Chi and Epstein, 2002; Noll, 1993).

Pax genes play an important role during organogenesis in many structures such as the ear, thymus, kidney, pancreas, nervous system and eyes (Dahl et al., 1997; Ton et al., 1991). Their products are defined by the presence of a paired-domain (PD), a highly conserved 128 amino acid DNA binding domain. The PD is organized as two independent sub domains, the amino-terminal PAI and the carboxy-terminal RED domain. These domains can bind to DNA, independently or synergistically (Czerny et al., 1993). The PAI and RED domains are always found together except in the *Pax-6*-like *Drosophila* protein Eyegone (Eyg) which lacks the PAI domain. Pax proteins often contain another DNA binding domain, a paired-type homeodomain (HD). For all Pax proteins, the 60 amino acid DNA-binding HD is characterized by a S₅₀, and these proteins can bind as homeo- or heterodimers with any paired-class HD to a palindromic DNA sequence (Wilson et al., 1995). Pax DNA-binding domains can also function through protein-protein interactions and via intramolecular assembly modified activity (Plaza et al., 2001) Between the PD and HD some Pax proteins also have an octapeptide motif (Breitling and Gerber, 2000; Treisman et al., 1991).

Pax-6

The *Pax-6* gene belongs to the gene family encoding paired-box transcription factors. The Pax-6 protein has a PD and a HD with no octapeptide (Callaerts et al., 1997). The gene was first isolated in human, mice and zebrafish but has also been found in many other animal species like chicken, quail, and *Drosophila* (Martin et al., 1992; Püschel et al., 1992; Qiring et al., 1994; Ton et al., 1991; Walther and Gruss, 1991). Its functional importance was revealed in part from

studies of the human eye condition aniridia caused by haploinsufficiency of the *Pax-6* gene and its homologous mouse phenotype, *Small eye (Sey)* which was confirmed to be caused by mutations in the same gene (Hill et al., 1992).

In vertebrates, *Pax-6* is expressed from the earliest stages of eye morphogenesis in the optic vesicle, which will grow in contact with the head ectoderm, giving rise to the lens placode. The lens placode will eventually invaginate and form the lens vesicle that will differentiate to the adult retina. Parallel to these events, the optic vesicle folds inward on itself, forming the optic cup which will develop into the neural and pigmented layers of the retina (Wawersik and Maas, 2000). However, *Pax-6* is not only expressed in the developing eye but also in the nasal epithelium, in specific regions of the brain and the spinal cord (Gehring and Ikeo, 1999).

As previously mentioned mutations in human and mouse confirm that *Pax-6* plays a critical role in eye formation. Iris and fovea hypoplasia is found in patients suffering from aniridia syndrome (Glaser et al., 1992) and smaller eyes with corneal and lenticular abnormalities in the *Sey/+* mice (Hill et al., 1992). The phenotype caused by *Pax-6* loss of function mutants (*Small eyes*) is strangely also observed in transgenic mice over expressing *Pax-6*, meaning that a precise dose of the protein is essential to ensure normal function (Schedl et al., 1996).

Homozygous mutations of *Pax-6* in humans is lethal resulting in loss of one or both eyes, nasal reduction and central nervous system defects (Glaser et al., 1994). In mice, homozygosity is also lethal, with embryos completely lacking eyes and nose and exhibiting severe brain damage (Hogan et al., 1986).

The identification of *eyeless* as a *Pax-6* homologue in *Drosophila* (Quiring et al., 1994) showed that *Pax-6* have an important function also in the development of insect eyes. In *Drosophila*, there is a second *Pax-6* gene, named *twin of eyeless (toy)* (Czerny et al., 1999). This is believed to be caused by a duplication event that occurred during insect evolution. So far, only in holometabolous insects, like *Drosophila* and *Bombyx*, have a second *Pax-6* gene been found and

there is no other animal phyla besides insects that have been shown to have more than one *Pax-6* gene (Gehring and Ikeo, 1999). Studies using these two *Pax-6* paralogs in the fruit fly have helped to identify genes cooperating with *Pax-6* in patterning of the fly eye and based on the conservation theory between flies and vertebrates, homologs of these genes have been cloned and their roles in vertebrate eye development examined (Georgala et al., 2011).

eyeless and *twin of eyeless*

Compared to vertebrate Pax-6 proteins, both Ey and Toy have a 90% sequence similarity in their homeodomain, while in their paired domain, Ey is more closely related to vertebrate Pax-6 than Toy (95% vs. 91% sequence identity). On the other hand, Toy shows significant sequence conservation in the C-terminal region with other Pax-6 proteins, which makes Toy overall more similar to Pax-6 proteins of other animal phyla (Czerny et al., 1999; Quiring et al., 1994).

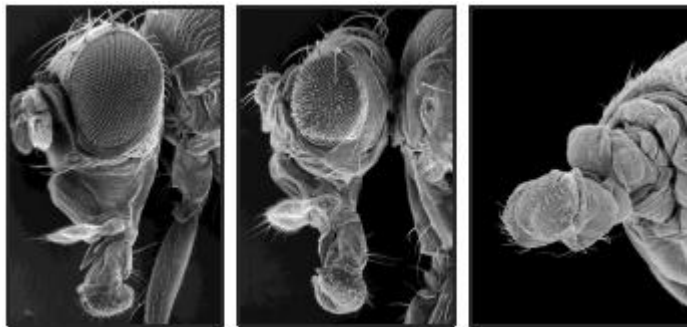


Figure 4. Head of a wild type vs. *toy*^{hdl} mutant flies.

Left picture showing a wild type fly with normal eyes. The middle picture shows a milder form of a *toy*^{hdl} mutant, where the fly has a smaller eye than the wild type. Right picture showing a strong mutant of *toy*^{hdl}, where almost the whole head is missing and the only part protruding from the body is the proboscis (Photos by Jesper Kronhamn).

Both *toy* and *ey* have important functions during eye development. Mutants of both genes give a variety of eye and head phenotypes with the strongest giving rise to an almost headless phenotype

meaning that they lack all structures derived from the eye-antennal disc (Figure 4) (Kronhamn et al., 2002)

The expression patterns of *toy* and *ey* are very similar in the developing visual system with the exception that *toy* is expressed already in the cellular blastoderm stage in a region covering the posterior procephalic area from where the embryonic eye-antennal primordia originate. (Czerny et al., 1999; Green et al., 1993; Younossi-Hartenstein et al., 1993). The expression of *toy* is confined to the head region throughout gastrulation and after germband retraction; it is detected in both the embryonic visual primordia in the head as well as in the ventral nerve cord (VNC) (Czerny et al., 1999).

ey is first detected during late germband extension where it is expressed in every segment of the VNC. Only a few cells in the brain are positive for *eyeless* expression at this point and compared to *Toy*, its expression remains more regionalized in the brain hemispheres during further development. *Ey* is also seen in the embryonic eye-antennal primordia (Czerny et al., 1999; Quiring et al., 1994).

When the eye-antennal imaginal disc is formed in early first instar larvae, all its cells express the eye selector genes *toy* and *ey* (Czerny et al., 1999). During second larval stage, the expression of *ey* and *toy* become restricted to the posterior part of the eye disc. This *toy* / *ey*-expressing region will give rise to the eye and the ocellar primordia, while the anterior region will form the antenna and the maxillary palp (Kenyon et al., 2003). During the third instar larval stage both genes continue to be transcribed throughout the undifferentiated part of the eye discs that lies anterior to the morphogenetic furrow and the proteins are also found in defined regions of the brain. (Czerny et al., 1999; Quiring et al., 1994).

Although the expression of *toy* and *ey* are similar during visual system development, the fact that targeted expression of either *Ey* or *Toy* can induce ectopic eye formation without induction of the other gene (Czerny et al., 1999; Punzo et al., 2004) indicates that the two *Pax-6* genes act in parallel and have redundant functions in eye development. This is not the case, however they can partially

substitute for each other with regards to head structures and survival (Jacobsson et al., 2009).

Mutant analysis and knock-down experiments indicate that *toy* is more important for development of head vertex (Kronhamn et al., 2002) and ocelli and in the latter case, *Toy* cooperate with *Otd* to activate *so* expression leading to bigger ocelli and sometimes ectopic ocelli (Blanco et al., 2010). *ey* is more important for compound eye development (Brockmann et al., 2011; Punzo et al., 2002; Seimiya and Gehring, 2000).

eyegone and twin of eyegone

The two *Pax-6*-like genes *eyegone* (*eyg*) and *twin of eyegone* (*toe*), are also the results of a duplication event (Aldaz et al., 2003; Jun et al., 1998). Both encoded proteins have a truncated Paired domain and bind DNA through the RED subdomain and the homeodomain (Rodrigues and Moses, 2004). They are expressed in the embryonic eye primordium and loss-of-function mutants lack eyes and have a headless phenotype similar to *ey* and *toy* (Dominguez et al., 2004; Jang et al., 2003; Yao et al., 2008).

In larval development, both *eyg* and *toe* are expressed in the eye-antennal disc anterior to the morphogenetic furrow (MF) in a narrow domain of cells along the dorsal-ventral compartment boundary and unlike the *Pax6* genes *toy* and *ey*, they do not extend laterally (Dominguez et al., 2004). Despite their almost identical pattern throughout development, *eyg* and *toe* have non-redundant roles in the eye where they use different combinations of proteins to influence retinal development (Jang et al., 2003; Yao et al., 2008).

***Drosophila* visual system**

The adult visual system of *Drosophila* consists of a pair of compound eyes and three simple eyes, called ocelli, located on the top of the

head vertex. Both types of eye structure develop from the embryonic eye primordia (Green et al., 1993).

During late embryogenesis, the two *Pax-6* genes: *toy*, *ey*, and the *Pax-6*-like genes, *eyg* and *twin of eygone* (*toe*) specify the eye primordia. However, proliferation and specification of the eye is triggered by the activation of other retinal determination genes during later larval stages (Yao et al., 2008).

The eye-antennal disc

The eye-antennal discs derive from the acron and several embryonic head segments, particularly the antennal and maxillary segments but the intercalary, labial and mandibular segments also contribute to the developing eye-antennal discs (Younossi-Hartenstein et al., 1993). In a dorsal view during late embryogenesis (stage 16), these segments can be seen as a v-shaped structure with the tip positioned between the brain hemispheres and are referred to as the embryonic eye-antennal primordium (Younossi-Hartenstein et al., 1993).

In the first instar larvae, the embryonic eye-antennal primordium develops into two eye-antennal discs. These discs consist of about 70 cells and continue to proliferate throughout larval life. They are club-shaped structures consisting of a narrow anterior antennal primordia and a broader posterior eye primordia. In the first and second instar, the eye-antennal discs are an undifferentiated epithelium of cells. During the third larval instar, the epithelium has grown into approximately 1600 cells and will start to differentiate. Cells at the posterior margin of the disc begin to organize into ommatidial precursors, due to a number of signaling events and the regulatory relationship between eye specification genes and patterning genes. Together these gene products will start a wave of cellular changes called the morphogenetic furrow (MF) to sweep over the disc leaving behind differentiated cells. As the furrow moves forward it produces a new column of ommatidia about every 2 hours (Heberlein and Treisman, 2000) By the end of metamorphosis the eye-antennal discs

have developed into four distinct organs (eyes, antenna, maxillary palps and ocelli) and the surrounding head cuticle in the adult fly (Figure 5) (Jones and Moses, 2004; Younossi-Hartenstein et al., 1993).

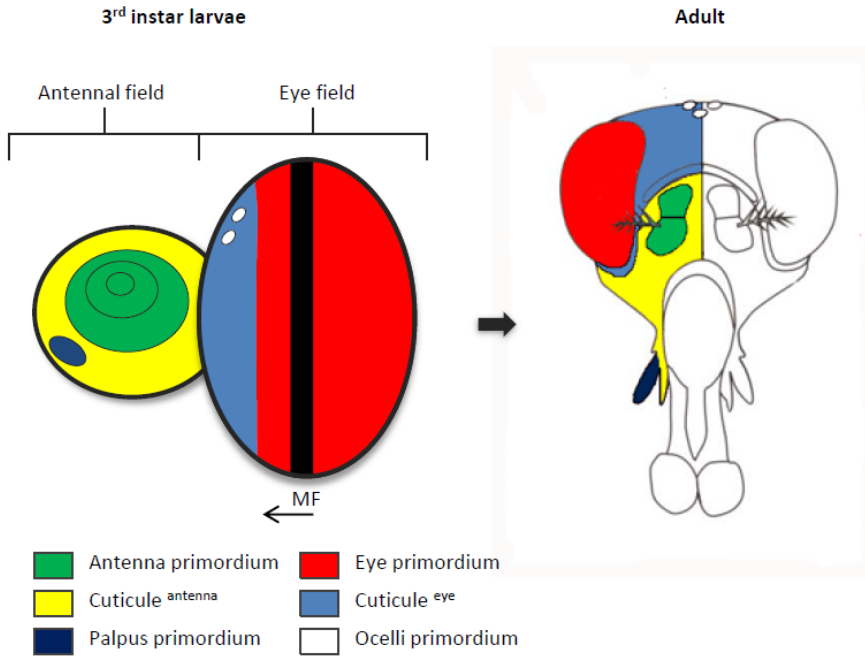


Figure 5. Fate map of an eye-antennal disc from 3rd instar larvae and corresponding structures of the adult head of the fly. Disc is shown with anterior to the left. Black stripe corresponds to the morphogenetic furrow (MF) Small arrow indicates MF movement. (Modified from Kenyon et al. 2003).

The adult eye

The adult compound eye consists of approximately 750 ommatidia and each ommatidium is like a unit eye with its own lens. This unit eye is an assembly of 14 cells: eight photoreceptors, four cone cells and two pigment cells. A precise arrangement of these cells makes each unit eye an exact copy of its neighbors (Heberlein and Treisman, 2000; Ready et al., 1976).

Eye specification and patterning

During larval development, a group of conserved tissue-specific genes controls the initiation of eye development in the eye-antennal discs of *Drosophila*. These genes function in a complex genetic regulatory hierarchy called the retinal determination (RD) network. The interactions in this network do not necessarily occur uniformly throughout the eye, but rather seems to be influenced by spatial and temporal considerations (Salzer and Kumar, 2009).

In *Drosophila* there are at least ten genes included in the RD network, *twin of eyeless (toy)*, *eyeless (ey)*, *eyegone (eyg)*, *twin of eyegone (toe)*, *eyes absent (eya)*, *sine oculis (so)*, *optix (opt)*, *dachshund (dac)*, *homothorax (hth)* and *teashirt (tsh)*. (Bessa et al., 2002; Bonini et al., 1993; Cheyette et al., 1994; Czerny et al., 1999; Kumar and Moses, 2001b; Mardon et al., 1994; Quiring et al., 1994).

Initially, *toy* and *ey* are expressed first in combination with the *Pax6*-like genes *eyg* and *toe*, in the eye-antennal primordia during late embryogenesis (Czerny et al., 1999; Yao et al., 2008). *Toy* activates *ey* transcription by binding to an eye specific enhancer of the *ey* gene in the embryo and both *toy* and *ey* activates the downstream target genes *eya* and *so* (Blanco et al., 2010; Halder et al., 1998; Niimi et al., 1999). *Ey* and *Toy* expression is maintained in the eye primordia onwards until differentiation.

During early second larval instar, *Eya* is expressed in the area of the eye disc that develops into the eye (Kenyon et al., 2003). *Eya* together with *So*, which is expressed slightly later in a pattern that fully overlaps that of *Eya* are considered to be the first marker for retinal determination. These genes form a protein complex which in turn regulates the upstream gene *ey* and the downstream gene *dac* (Pappu et al., 2005; Pauli et al., 2005; Pignoni et al., 1997). During third instar larva stage, both *Eya* and *So* are expressed at high levels within and posterior to the MF, and in front of the furrow they are expressed in a gradient with decreasing expression near the antennal disc (Bonini et al., 1993; Cheyette et al., 1994). *so* is required for the development of the entire visual system, including the compound eyes, the ocelli, the optic lobe of the brain and the larval eye

(Cheyette et al., 1994; Pignoni et al., 1997). *eya* is also required for formation of compound eyes and ocelli, since *eya* mutants lack both visual systems (Zimmerman et al., 2000).

dac is so far the most downstream member of the RD network, the protein distribution in front of the MF overlaps that of So and Eya. Posterior to the furrow *dac* expression is maintained in approximately eight rows of cells and then quickly disappears. Null-mutants of *dac* develop with dramatically reduced or absent eyes and altered expression of the gene is sufficient to induce ectopic eye development in non-retinal tissue (Mardon et al., 1994; Shen and Mardon, 1997). Because of their regulatory interactions, *ey*, *eya*, *so* and *dac* are known as the “core retinal genes”, since their co-expression is necessary to lock-in the eye fate within the eye field (Chen et al., 1997; Kenyon et al., 2003; Kumar and Moses, 2001a; Pappu and Mardon, 2004).

In addition to the core network of genes, other factors interact with these genes and are also required for early eye development or can induce ectopic eye formation.

tsh and *hth* are two transcription factors that are expressed in the developing eye discs. The most anterior domain in the eye field, which is next to the antennal portion of the eye-antennal imaginal disc, expresses Hth but not Tsh. In a posterior domain, both of these genes are co-expressed but are then repressed behind the MF (Bessa et al., 2002). Tsh overexpression in the eye disc can induce ectopic eye development and together with Hth and Ey, Tsh maintains the eye disc cells in a proliferative and undifferentiated state (Singh et al., 2002).

optix is a member of the *six/so* gene family in *Drosophila*. The protein contains both a six domain and a homeodomain. Targeted expression of the protein induces ectopic eye structures in the antenna and head, but not in wings or legs. The efficiency of inducing ectopic eyes is rather low compared to that of *ey*, 20% vs. 100%. So far, no loss of function mutants for *optix* is available making its functional role in the eye disc rather uncertain (Kenyon et al., 2005; Seimiya and Gehring, 2000).

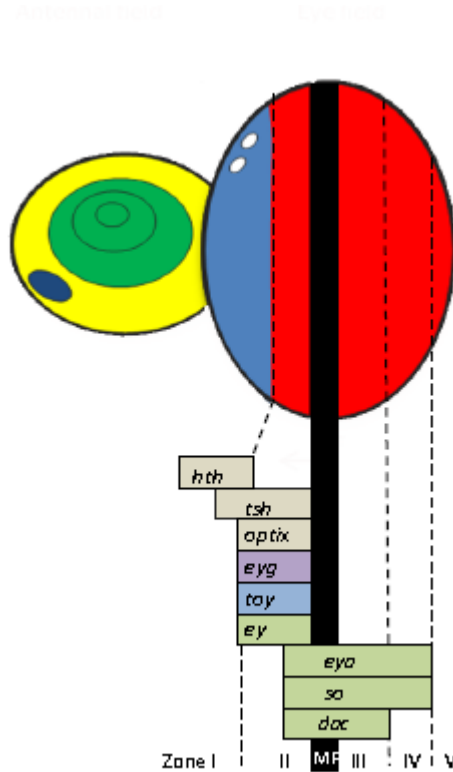


Figure 6. Schematic drawing of a third instar eye-antennal imaginal disc. The expression zones are modified after data presented in (Bessa et al., 2002).

Based on where the eye specification genes are expressed in the eye, the third instar eye antennal disc can be divided into five zones, where zone 1 corresponds to the anterior region of the eye and express *hth* and *tsh*. In zone 2, extending to the MF, all of the eye specification genes are expressed, except *hth*. Zone 3 is posterior to the MF and the expression of *dac* is seen here together with *So* and *Eya*. Zone 4 begins where *dac* expression ceases and extends to the posterior edge of the eye field. The fifth zone corresponds to the posterior margins of the eye field (Figure 6) (Salzer and Kumar, 2009).

In addition to the RD genes, extracellular signaling molecules such as Notch (N), Hedgehog (Hh), Decapentaplegic (Dpp) and Wingless (Wg) are required for coordinating growth, proliferation, patterning and cell fate specification during retinal morphogenesis in *Drosophila*.

(Firth and Baker, 2009; Kenyon et al., 2003; Pappu and Mardon, 2004).

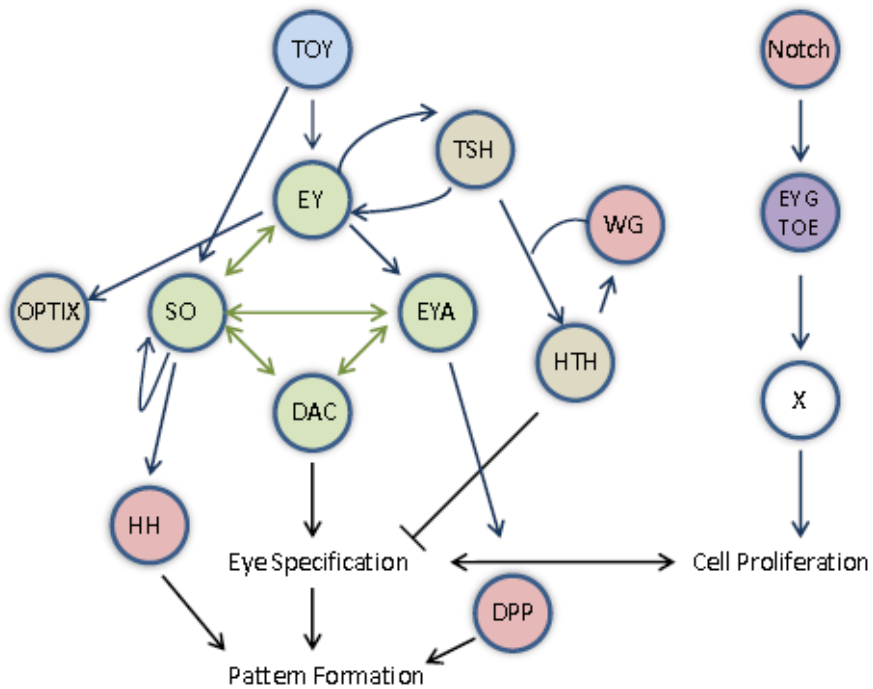


Figure 7. Schematic diagram of the retinal determination network

Modified from (Salzer and Kumar 2009).

Hedgehog and Dpp at the posterior margin of the disc are necessary for the definition of the eye disc primordia, as they activate expression of *eya* and *so*. Both genes are required for initiation of the morphogenetic furrow, leading to photoreceptor differentiation (Domínguez and Hafen, 1997; Pappu and Mardon, 2004).

wg is expressed at the lateral margins just anterior to the advancing furrow, ectopic expression of *wg* in the furrow halts its progression and loss of *Wg* at the margins induces ectopic furrow and retinal development. This indicates that *wg* is a negative regulator for eye development and it is repressed at the posterior compartments by Dpp (Domínguez and Casares, 2005; Treisman and Rubin, 1995).

Notch (N) is a transmembrane receptor protein that promotes growth of the eye disc, which is essential for the emergence of an eye primordium. In Notch loss-of-function mutants, the onset of *eya* is delayed and there is a reduction in eye size (Dominguez et al., 2004; Kenyon et al., 2003). N signaling also regulates *eyg* expression in the eye disc. Down regulation of N results in reduction of *eyg* expression while activation of N induces *eyg* (Dominguez et al., 2004). N regulation of *eyg* seems only to occur in the second larval stage, since in the third larval stage expression of *eyg* is turned off by the processing MF (Wang et al., 2008).

***toy* and *ey* in brain and ventral nerve cord**

Most studies of *ey* and *toy* have focused on their role in eye development but both genes also show strong expression in the brain and ventral nerve cord (VNC) indicating a role in *Drosophila* central nervous system development (Czerny et al., 1999; Kammermeier et al., 2001; Quiring et al., 1994).

The brain of *Drosophila* is organized in three different neuromeres: protocerebrum, deutocerebrum and tritocerebrum. The protocerebrum is the largest part of the brain and is dominated by the optic lobes, mushroom bodies (MB) and the central complex neuropils. These neuropils are important for integration of olfactory and visual stimuli, learning and memory as well as control of locomotion (Callaerts et al., 2001).

Both *ey* and *toy* are expressed in all three neuromeres of the brain. *ey* is expressed mostly in the dorsal domains of these neuromeres, whereas *toy* is expressed in the medial and ventral domains (Kammermeier et al., 2001).

In the embryonic VNC, both *Ey* and *Toy* show a metameric pattern, however mostly in different sets of neurons since they are only co-expressed in a small number of cells (Furukubo-Tokunaga et al., 2009). This non-overlapping relation that occurs in the brain and VNC compared to eye primordia suggests that the genes are independently controlled by different sets of regulatory factors in the

specification of different sets of neurons in the brain. Analyses of mutants of *toy* and *ey* further confirm this, since loss of Toy causes deformation of the embryonic brain hemispheres and in the major axonal tracts, whereas Ey is downregulated only in severe cases but not in the MB primordia. Ey was only barely affected in the absence of Toy in milder mutants (Furukubo-Tokunaga et al., 2009; Kammermeier et al., 2001).

In the larval brain, Toy is observed in the various regions of the central brain including the mushroom bodies (MB) and the optic lobes. Ey is strongly expressed in the MBs but only moderately in the central brain and a part of the medulla (Furukubo-Tokunaga et al., 2009).

AIMS OF THIS THESIS

The aims of this thesis were to investigate how the transcription of the *toy* gene is regulated and how Ey and Toy proteins interact in the development of eyes.

- What sequences are important for the correct expression of *toy* and in what way are *ey* and *toy* able to substitute for each other during eye development.
- Identify possible upstream regulators of the *toy* gene
- Investigate if there are any *toy* specific enhancers located within the intron sequences of the gene.

Paper I

The *Drosophila Pax-6* paralogs have different functions in head development but can partially substitute for each other

Pax genes are transcription factors that have important roles during development of the central nervous system and brain, as well as of the peripheral nervous system and sensory organs (Chi and Epstein, 2002). In *Drosophila*, the two *Pax-6* genes *eyeless* (*ey*) and *twin of eyeless* (*toy*) are located high up in the genetic hierarchy controlling eye development.

toy and *ey* are expressed in overlapping patterns during embryonic eye development and in larval eye-antennal imaginal discs, but not in the embryonic ventral nerve cord (VNC) (Kammermeier et al., 2001). Since both genes give rise to ectopic eyes when misexpressed (Czerny et al., 1999; Halder et al., 1995) and strong mutants in both genes can produce similar headless phenotypes (Kronhamn et al., 2002), we investigated in what way these genes can substitute for each other.

We made *toy* driver lines to be able to express *toy* and various reporter genes in a *toy*-specific pattern by dissecting the 5' upstream cis-regulatory region of the *toy* gene. We found that a 1300bp region surrounding the *toy* transcription start was sufficient to drive reporter gene expression in the embryonic eye- antennal primordium and brain as well as in larval eye-antennal discs. When using this region to drive a UAS-RNAi transgene, flies with a headless phenotype similar to *toy* null mutants were obtained.

Rescue experiments were set up to test for possible redundancy between *toy* and *ey*. For this, we used transgenic lines expressing Gal4 under the *toy* 5' regulatory region generated or the eye-specific enhancer from the *ey* gene (Bonini et al., 1997; Hauck et al., 1999) to drive *UAS-Pax-6* genes, analyze the viability, and head phenotype of the rescued flies. We found that exogenous Toy could significantly rescue both *toy* and *ey* homozygous mutants to viability. The pupal

lethality of *toy*^{hdl} and *toy*^{G7.39} mutants were rescued by expression of Ey. Partial rescue of the gene-specific eye phenotypes was also evident. Furthermore, we showed that inhibition of apoptosis increased survival of *toy*^{hdl} homozygotes, but did not improve eye phenotypes.

We show that overexpression of either *toy* or *ey* respectively can rescue lethality of homozygous *ey*^D mutants, also suppress the *ey*^D phenotype, and partially restore head development.

We also found an extreme temperature-dependent variability in the phenotypic manifestation of the *toy*^{hdl} mutant stock. At higher temperatures, the mutant phenotype is more severe, possibly due to a more rapid development and a subsequent low activation of target genes takes place. We find that it is only possible to rescue *toy*^{hdl} mutants from lethality at lower temperatures (15 to 22). At 25 degrees we find that *toy* mRNA levels are elevated in *toy*^{hdl} flies carrying the *UAS-toy* construct and that this increase in Toy expression levels is lethal.

Paper II

Empty spiracles is repressing *twin-of-eyeless* in the *Drosophila* embryonic head

The visual system of *Drosophila* originates from a field in the dorsal neuroectoderm in the head region of the embryo. This field will later develop into the eye-antennal discs in the larvae. The specification of the eye-antennal disc primordium requires the expression of two paralogous *Pax-6* genes: *twin of eyeless* (*toy*) and *eyeless* (*ey*). Toy is first expressed in a region overlapping the visual anlagen in the early embryo and is later found in the brain and ventral nerve cord as well as in the eye primordium of the eye-antennal discs. (Czerny et al., 1999). Ey is induced by Toy and expression is completely overlapping in the eye-antennal primordium in the embryo.

toy is considered the first eye specification gene expressed during eye formation and activates *eyeless*. What regulates *toy* expression is still unclear. Toy is not activated by any known feedback system, which sets it apart from the rest of the eye specification network

(Czerny 1999). However, a model has been proposed for activation of the first *toy* expression by maternally deposited products from the genes *bicoid*, *torso* and *dorsal* (Blanco and Gehring, 2008).

To shed new light on *toy* regulation, we focused on the embryonic eye-antennal primordium and searched for early head specific genes that- as homozygous mutants- alter the expression pattern of Toy.

Immunohistochemical analysis on *ems* mutant embryos revealed an alteration in the Toy expression pattern in the eye-antennal primordium in such a way that it was broadened laterally and towards the midline. These findings gave us a first indication of a regulatory relationship where *ems* might work as a repressor of *toy* in the eye primordium. As a further test of our hypothesis of a regulatory relationship between *ems* and *toy*, we performed clonal analysis, inducing ectopic expression of Ems in somatic clones in wild-type eye discs. When Ems positive clones appeared at the margin of the antennal field or in the anterior dorsal part of the eye field (the dorsal head capsule primordia), the expression of Toy was severely reduced or absent. Ectopic analysis with Toy and EMS showed that Ems represses ectopic Toy expression also at later developmental stages and that Ems does not repress *eyeless* expression.

Our overall conclusion is that Toy attains a broader expression region in the eye primordia of *ems* mutant embryos and that ectopic expression of *ems* blocks Toy expression in eye-antennal discs. We thereby conclude that the function of Ems is to restrict the region of the eye anlagen

Paper III

Identification of regulatory regions within the intron sequence of the *toy* gene.

In *Drosophila*, the two *Pax-6* genes *eyeless* and *twin of eyeless* share a similar expression pattern in the brain and ventral nerve cord but are not activated at the same time in these regions. Despite their similar expression pattern, there are hardly any overlap between the proteins in brain and ventral nerve cord (Czerny et al 1999,

Kammermeier et al. 2001). Since both genes are expressed in several tissues at different time points in development, one might expect that there are more than one enhancer regulating their expression. Previous studies have shown that there are binding sites for regulatory proteins within the introns of the *eyless* gene and in vertebrate *Pax-6* genes (Williams et al. 1998). Whether this also is true for *toy* is still not known.

In this paper, we investigate presumptive *toy* enhancer regions within the intron sequences of the gene. We made four constructs, each containing an 851bp region around the transcription start, which included the 5'UTR and together with different intron sequences, this was fused to a β -galactosidase coding gene. One construct only contained the 851bp sequence and was used as a control.

Immunohistochemical analysis on transgenic embryos with our Toy antibody (Jacobsson et al. 2009) together with a β -galactosidase antibody reveal that there might be an enhancer region driving *toy* expression in embryonic ventral nerve cord within intron 2.

Paper I

Our investigation of the cis regulatory region of Toy showed that a 1300bp sequence surrounding the transcription start site was sufficient to give rise to expression in both the embryonic eye-antennal primordium and in the larva eye-antennal disc. However, in our transgenes there was not a complete restoration of the endogenous expression of Toy indicating the role of more than one enhancer. In 2010, Blanco and coworkers found an enhancer region located further upstream from the area of our investigation. This enhancer seems only to be embryo specific since there was no expression induced in larval structures. However, a more comprehensive analysis of the 1300bp region could be of interest in the future, to identify smaller enhancer regions responsible for embryonic and larval expression respectively and possible binding of cis acting protein candidates.

In the most severe *toy*^{hdl} mutants, all expression of *toy* is gone from the embryonic eye-antennal primordium and from all the structures derived from the eye-antennal discs (Kronhamn et al. 2002). The variations in mutant phenotypes with both *eyeless* and *toy*^{hdl} has puzzled us. The critical period for inducing the headless phenotype in *toy*^{hdl} mutants is during embryonic stages 12-16 (Kronhamn et al. 2002). Therefore, we have hypothesized that the defects seen in the eye-antennal discs of *toy* mutants are initiated during the time when the embryonic eye-antennal primordium is formed. In the absence of Pax-6, the eye primordium begin to form, but do not develop into an eye, since the eye developmental program is not initiated. However, in some *toy* loss-of-function, mutants there sometimes are found red pigment cells or facets inside the thorax and this was believed to be an effect of a still functional *eyeless* gene. It has now been shown that in *toy* and *ey* double loss-of-function mutants, this pigment cell cluster or facets inside the thorax still appear (Gehring and Seimiya 2010). Apparently this is due to the action of *eyegone*, another Pax6-like gene, that also is expressed in the embryonic eye primordium. Its function is to promote cell proliferation in the early eye disc and to

promote eye development by suppression of *wingless*. By making a triple loss-of-function mutant with *ey*, *toy* and *eyg*, no eyes in the thorax is formed (Gehring and seimiya 2010).

Misexpression of *toy* leads to the induction of ectopic eyes in *eyeless* null mutant background (Punzo et al. 2004). We show that homozygous *eyeless* loss-of-function mutant flies can be fully rescued by increased *toy* transcription, without any accompanying increase in *ey* expression. Therefore, we conclude that Toy can promote eye and head development in an Ey-independent manner, which has also been suggested earlier (Punzo et al. 2004). One thing that could be of interest here is to see whether this has something to do with Eyg.

The Ey^D protein lacks the entire homeodomain and 660 amino acids in the C-terminus, giving the dominant *eyeless* phenotype (Kronhamn et al. 2002). Our theory that the dominance of the *ey*^D allele is due to a dominant negative function of the Ey^D protein has been supported by the finding that Df(4)J2, which is a large deletion uncovering *ey*, *toy* and *spa* (*Pax2*) genes, has completely normal eyes in heterozygous condition (Gehring and Seimiya 2010).

Paper II

We found that overexpression of the gap gene *ems* together with *toy* block ectopic Toy expression in the wing, leg and halter discs leading to loss of neuron formation. To try to verify this as a direct interaction of Ems on *toy*, we made overexpression clones in the eye disc of larvae. Clones positive for Ems lacked Toy expression confirming the theory of a direct effect. To rule out possible effects of Ems on *eyeless* we made ectopic expression with both genes in the same way as with *toy and ems*, and found that no block in neuron formation was achieved. The *ems* repression on *toy* could also possibly be indirect, mediated by protein-protein interactions or internal regions located in the *toy* exons.

Paper III

In this paper, we found a potential enhancer element within the second intron of the *toy* gene. However, further investigations are needed to fully prove that this region has an enhancer function. First, we do not seem to have found the whole region since the expression pattern is not complete, meaning that a wider search and more transgenic lines have to be made.

One thing that speaks for the possibility of this being a functional enhancer is the finding of protein binding hot spot regions in the second intron of the *toy* gene (modENCODE). Whether or not these protein-binding sites have any regulatory role in *toy* is not yet investigated.

So far, these constructs have only been investigated for expression in embryo tissue but it would also be interesting to see whether these constructs give any expression in larval tissues, like the eye-antennal disc or the brain.

ACKNOWLEDGEMENTS

Då har man kommit till slutet då...eller början, beroende på hur man väljer att se på saker och ting. Att jag har kommit så här långt är ju såklart inte bara min egen förtjänst utan har många personers inblandning, en del för rent praktiskt deltagande och andra kanske på ett mer själsligt plan. Hur som helst så vill jag säga tack till Er.

Först och främst min handledare **Åsa Rasmuson-Lestander**, för att jag fick chansen att pröva mina vingar i ditt lab. För att du har gett mig frihet under eget ansvar och låtit mig utvecklas. För din otroliga kunskap inom genetik och för att du stöttat mig under alla år på vägen mot målet.

Speciellt stort tack till de som utgjort kärnan i grupp Å.R-L, åtminstone som jag känner den:

Sa Chen – Thanks for all the help throughout the years. Having you as some sort of laboratory dictionary has been much appreciated and helped a lot many times. Thanks also for your kindness and warmth, all dinners and nice events. I am really glad that you only moved to the next corridor☺

Anna Larsson- Annis, Jag har verkligen saknat våra dagliga samtal på labbet, om ditten och datten och allting och ingenting. Tur att det finns Skype, även om det inte alls är som IRL. Lycka till med allt du gör, vi ses. Ps. Jag ser fram emot den där öde ön nån gång efter åtti....;)

Erik Tegeling - Mr. choklad/cola/smyga tyst på tåna mannen!! Jag uppskattar alla intressanta diskussioner vi haft genom åren och med tiden har jag nog, öhhhh...., även lyckats förstå vad de handlade om ;) Lycka till med allt i livet...och sänk inga fler kanoter!!!

John Skottheim Honn – Det har varit otroligt uppskattat med en till på *toy* projektet. Tack för all hjälp och trevliga diskussioner om både forskning och annat. Tack också för att du har koll på viktiga arbetsplatsrutiner och att du styr upp fikagruppen, väntar med spänning på "Coffee stain Collection of recipes" Lycka till i fortsättningen...

Karin Ekström- För trevliga diskussioner om allt mellan himmel och jord, för excellent hjälp med korsningar och omhändertagande av flugstammar, Tack!

Chaurui Li – you are the first person I meet that took slalom skis and casting rod, when going on ice fishing at Kont. Perhaps now you know that it is a Big difference between slalom and cross-country skis ;) Good luck in the future.

Thanks also to all the former people in our lab that has come and gone throughout the years: **Kerstin K, Björn, Alex, Denise, Jesús, Lu, Anna .S och Tove.**

My friends at the department that has given me many joyful moments and a lot of laughs both on the inside and outside of work.

Chaz- Thanks for all the craft nights, nice dinners and fun parties. Thanks for showing me some American traditions and for comments on my writing. I wish you the very best for the future and good luck with the “drunken bead” I will certainly drop in some time ;).

Linus- Tack för alla skratt och roliga konversationer, för att du låtit mig invadera hemmet med diverse ”crap”, eller jag menar craft-stuff. Det kan ju tänkas att det inte lyckas så bra med ”my wifes crap” men då kan vi ju alltid göra en serie med ”Conny och Anna” och deras liv i grus gropen. Istället för ”solsidan” kan det bli ”baksidan”, det måste ju vara en given succé 😊

Lina- Tack för roliga tider med pysselkvällar, kräftfest i stugan, filminspelningar och fester. Jag önskar dig all lycka med din framtida forskning och livet.

David Gunnarsson- man säger ju att ett gott skratt förlänger livet, i så fall har du nog förlängt mitt flera decennier med dina roliga historier och galna olycksdrabbningar. Lycka till i allt du gör och glöm inte 2022!!

Regina Ulfsparre – för alla skratt, du är ju den okrönte ”godornas mästare”, hur roligt har det inte varit genom åren. Tack också för din fina vänskap, positiva sätt och för att du alltid bjuder på dig själv.

Sara – Tack för alla roliga stunder med filminspelningar och för att du är en mästare på manus och regi, kanske man borde anlita dig till ”baksidan”.

Anna-Mia – för trevliga pratstunder och roliga tider, med början på våra genetik studier.

Therese E – Välkommen tillbaka till Fly floor, hoppas det ger inspiration för framtiden.

Fredrik H - Du verkar alltid ha koll på det senaste inom forskning, tack vare det har jag fått några nya perspektiv på saker och ting. Tack också för att du uppskattar min "skådespelar talang" och glöm inte att det alltid finns en ledig plats för dig i fika gruppen om du skulle få abstinens på att baka 😊

Ava and Sajna – I really had fun during our teaching; I will always remember Ava's explanation about chromosome segregation...😊
Good luck in your future Phd-studies.

Hande- For always being happy and nice. I wish I was as daring when it comes to changing my hair 😊

Friday fika group and Lunch companions; everyday there is a new interesting discussion going on and sometimes the least most expected!**Caroline B,Isabelle,Kristoffer,Hande,Jessica,Viktorija,Sajna, Philge, Erik, John, Chaz, Linus, Margarida, Lina, Lisa, Sa, Linus**

Other people at the department:

Damerna på media, speciellt **Christina**, för att ni alltid är trevliga och sköter mycket av det som man bara tar för givet, Tack! **Flugmats köket**, för att ni kokar mat til våra älskade "husdjur"**Johnny**, för att vi helt enkelt inte klarar oss utan dig. **Mariella**, för att du alltid är trevlig och för att du håller snyggt på bygget! **Marek**, för värdefulla installationer. Alla sekreterare, speciellt **Helena** och **Ethel**, för värdefull hjälp i blankett djungeln.

Some people from the Old genetics:

Anssi Saura – För din otroliga vetenskapliga kunskap (och om en hel del annat också). Tack för att du alltid är omtänksam och får en att känna sig välkommen. Om alla vore som du så skulle världen definitivt vara annorlunda.

Helena Östbye- Tack för all hjälp med pappers excersisen, om det så har gällt föräldrapenning, semester, lön eller "traktamenten" så har du grejat allt på ett litet kick.

Jan Larsson – För inspiration i både forskning och undervisning. Du är en excellent undervisare och forskare.

Stefan A. Escher – alltid roliga och crazy Stefan. Jag tycker du ska komma hit lite oftare och hälsa på, det är för lite "befrielsefest" nuförtiden och ingen har ju nån aning om vilka "skålar" man bör ha och i vilken ordning de ska utbringas!!

Jesper Kronhamn – för introduktion till projektet och för trevliga pratstunder, för att vi fick komma och kolla på erat hus så att vi kunde bygga ett likadant ;)

To all the Fly floor people for creating a nice work atmosphere

Martin, Camilla, Jens-Ola, Caroline, Ruth, Yuri, Murat, Philge, Dan, Ingrid, Yaso, Sajna, Jesper, Fredrik, Barbara, Pelle, Margarida, Anna-Mia, Lina, Anders, Janne, Karin, Therese, Meir, Peter, Ines, Jana mm mm

Andra vänner och familj, som betyder väldigt mycket för mig

Hundträningsgänget och Riesenlaget, speciellt **Ingela N.** Ni har varit guld värda för mig under mina första år här i Umeå. Jag vet var jag ska hitta er när jag har en ny fyrbening...

Therese B - Finare och omtänksammare vän får man leta efter. Tur att jag har dig och att du lånar ut Leo till mig ibland. Tack för allt roligt vi har gjort genom åren, hoppas vi kommer att ha måååå fler att se fram emot.

Ralf – Det ska bli roligt att få lära känna dig mera.

Pernilla och **Tomas**- tack för alla middagar, utflykter och annat kul vi gjort genom åren, det har varit guld värt. **Pernilla**, jag håller faktiskt fullständigt med, jag skulle också ha valt Vin Diesel över Frank Andersson vilken dag som helst!!!... Allt mitt bästa till **Alva, Axel** och **Theo** också!

Sandra och **Mattias** - Stort tack för alla utflykter, bastukvällar, skoterturer, fester, spelkvällar ja, you name it. **Sandra**, tack för den fina bilden till avhandlingen och för initiativ till Ladies night, det har

verkligen varit ett behövt bråk och jag ser redan fram emot nästa gång. **Frida** – du är fantastisk!

Hasse och Kerstin- Tack för allt barnvaktande, middagar, husbyggande, trevliga resor och utflykter, det har varit mycket uppskattat och jag ser fram emot mera.

Tack **Mamma**, för att du är den positiva, den som alltid stöttar och tror på mig. Du är en stor förebild för mig i mitt eget föräldraskap och jag hoppas att jag blir en lika bra mamma som du, Jag älskar dig!

Pappa, du har gett mig min analytiska förmåga, mitt kritiska granskande och att alltid vilja göra allt själv. Du har lärt mig att bli självständig och att det man inte kan det kan man alltid läsa sig till!

Nina - min allra käraste syster. Delad glädje är dubbel glädje och delad sorg blir bara hälften så svår, vissa saker i livet hade varit så otroligt mycket värre om inte du funnits där och delat dem. Jag är så oerhört glad att du finns. Du är bäst och Jag älskar dig!

Fredrik – Att önskningar kan gå i uppfyllelse det är du ett levande bevis på, välkommen till familjen. Jag önskar er all lycka i världen.

”Det bästa som vi äga,
det kan man inte giva,
det kan man inte säga och inte heller skriva”
/ karin Boye

Magnus- Du är mannen i mitt liv. Tack för det stöd och den kärlek du ger mig, för att du tror på mig och gör mig starkare. Jag vet att vi kan klara allt tillsammans, Jag älskar dig!

Robin och Elin- Mina finaste gulltroll. Ni är viktigast i livet och min kärlek till er är oändlig.

REFERENCES

- Aldaz, S., Morata, G., and Azpiazu, N. (2003). The Pax-homeobox gene *eyegone* is involved in the subdivision of the thorax of *Drosophila*. *Development* *130*, 4473-4482.
- Bessa, J., Gebelein, B., Pichaud, F., Casares, F., and Mann, R.S. (2002). Combinatorial control of *Drosophila* eye development by *eyeless*, *homothorax*, and *teashirt*. *Genes Dev* *16*, 2415-2427.
- Blanco, J., Pauli, T., Seimiya, M., Udolph, G., and Gehring, W.J. (2010). Genetic interactions of *eyes absent*, *twin of eyeless* and *orthodenticle* regulate *sine oculis* expression during ocellar development in *Drosophila*. *Dev Biol* *344*, 1088-1099.
- Blanco, J., Seimiya, M., Pauli, T., Reichert, H., and Gehring, W. (2009). *Wingless* and *Hedgehog* signaling pathways regulate *orthodenticle* and *eyes absent* during ocelli development in *Drosophila*. *Dev Biol* *329*, 104-115.
- Bonini, N.M., Bui, Q.T., Gray-Board, G.L., and Warrick, J.M. (1997). The *Drosophila* *eyes absent* gene directs ectopic eye formation in a pathway conserved between flies and vertebrates. *Development* *124*, 4819-4826.
- Bonini, N.M., Leiserson, W.M., and Benzer, S. (1993). The *eyes absent* gene: genetic control of cell survival and differentiation in the developing *Drosophila* eye. *Cell* *72*, 379-395.
- Breitling, R., and Gerber, J.K. (2000). Origin of the paired domain. *Dev Genes Evol* *210*, 644-650.
- Brockmann, A., Domínguez-Cejudo, M.A., Amore, G., and Casares, F. (2011). Regulation of ocellar specification and size by *twin of eyeless* and *homothorax*. *Dev Dyn* *240*, 75-85.
- Callaerts, P., Halder, G., and Gehring, W.J. (1997). *PAX-6* in development and evolution. *Annu Rev Neurosci* *20*, 483-532.
- Callaerts, P., Leng, S., Clements, J., Benassayag, C., Cribbs, D., Kang, Y.Y., Walldorf, U., Fischbach, K.F., and Strauss, R. (2001). *Drosophila Pax-6/eyeless* is essential for normal adult brain structure and function. *J Neurobiol* *46*, 73-88.
- Chen, R., Amoui, M., Zhang, Z., and Mardon, G. (1997). *Dachshund* and *eyes absent* proteins form a complex and function synergistically to induce ectopic eye development in *Drosophila*. *Cell* *91*, 893-903.
- Cheyette, B.N., Green, P.J., Martin, K., Garren, H., Hartenstein, V., and Zipursky, S.L. (1994). The *Drosophila sine oculis* locus encodes a homeodomain-containing protein required for the development of the entire visual system. *Neuron* *12*, 977-996.

- Chi, N., and Epstein, J.A. (2002). Getting your Pax straight: Pax proteins in development and disease. *Trends Genet* 18, 41-47.
- Cohen, S., and Jürgens, G. (1991). *Drosophila* headlines. *Trends Genet* 7, 267-272.
- Cohen, S.M., and Jürgens, G. (1990). Mediation of *Drosophila* head development by gap-like segmentation genes. *Nature* 346, 482-485.
- Czerny, T., Halder, G., Kloter, U., Souabni, A., Gehring, W.J., and Busslinger, M. (1999). twin of eyeless, a second Pax-6 gene of *Drosophila*, acts upstream of eyeless in the control of eye development. *Mol Cell* 3, 297-307.
- Czerny, T., Schaffner, G., and Busslinger, M. (1993). DNA sequence recognition by Pax proteins: bipartite structure of the paired domain and its binding site. *Genes Dev* 7, 2048-2061.
- Dahl, E., Koseki, H., and Balling, R. (1997). Pax genes and organogenesis. *Bioessays* 19, 755-765.
- Dalton, D., Chadwick, R., and McGinnis, W. (1989). Expression and embryonic function of empty spiracles: a *Drosophila* homeo box gene with two patterning functions on the anterior-posterior axis of the embryo. *Genes Dev* 3, 1940-1956.
- Dominguez, M., Ferres-Marco, D., Gutierrez-Aviño, F.J., Speicher, S.A., and Beneyto, M. (2004). Growth and specification of the eye are controlled independently by Eye gone and Eyeless in *Drosophila melanogaster*. *Nat Genet* 36, 31-39.
- Domínguez, M., and Casares, F. (2005). Organ specification-growth control connection: new in-sights from the *Drosophila* eye-antennal disc. *Dev Dyn* 232, 673-684.
- Domínguez, M., and Hafen, E. (1997). Hedgehog directly controls initiation and propagation of retinal differentiation in the *Drosophila* eye. *Genes Dev* 11, 3254-3264.
- Finkelstein, R., and Perrimon, N. (1991). The molecular genetics of head development in *Drosophila melanogaster*. *Development* 112, 899-912.
- Firth, L.C., and Baker, N.E. (2009). Retinal determination genes as targets and possible effectors of extracellular signals. *Developmental Biology* 327, 366-375.
- Furukubo-Tokunaga, K., Adachi, Y., Kurusu, M., and Walldorf, U. (2009). Brain patterning defects caused by mutations of the twin of eyeless gene in *Drosophila melanogaster*. *Fly (Austin)* 3, 263-269.

- Gao, Q., and Finkelstein, R. (1998). Targeting gene expression to the head: the *Drosophila* orthodenticle gene is a direct target of the Bicoid morphogen. *Development* *125*, 4185-4193.
- Gehring, W., and Ikeo, K. (1999). Pax 6: mastering eye morphogenesis and eye evolution. *Trends Genet* *15*, 371-377.
- Georgala, P.A., Carr, C.B., and Price, D.J. (2011). The role of Pax6 in forebrain development. *Dev Neurobiol* *71*, 690-709.
- Glaser, T., Jepeal, L., Edwards, J.G., Young, S.R., Favor, J., and Maas, R.L. (1994). PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. *Nat Genet* *7*, 463-471.
- Glaser, T., Walton, D.S., and Maas, R.L. (1992). Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. *Nat Genet* *2*, 232-239.
- Green, P., Hartenstein, A.Y., and Hartenstein, V. (1993). The embryonic development of the *Drosophila* visual system. *Cell Tissue Res* *273*, 583-598.
- Grossniklaus, U., Cadigan, K.M., and Gehring, W.J. (1994). Three maternal coordinate systems cooperate in the patterning of the *Drosophila* head. *Development* *120*, 3155-3171.
- Halder, G., Callaerts, P., Flister, S., Walldorf, U., Kloter, U., and Gehring, W.J. (1998). Eyeless initiates the expression of both sine oculis and eyes absent during *Drosophila* compound eye development. *Development* *125*, 2181-2191.
- Halder, G., Callaerts, P., and Gehring, W.J. (1995). Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. *Science* *267*, 1788-1792.
- Hartmann, B., Reichert, H., and Walldorf, U. (2001). Interaction of gap genes in the *Drosophila* head: tailless regulates expression of empty spiracles in early embryonic patterning and brain development. *Mech Dev* *109*, 161-172.
- Hauck, B., Gehring, W.J., and Walldorf, U. (1999). Functional analysis of an eye specific enhancer of the eyeless gene in *Drosophila*. *Proc Natl Acad Sci U S A* *96*, 564-569.
- Heberlein, U., and Treisman, J.E. (2000). Early retinal development in *Drosophila*. *Results Probl Cell Differ* *31*, 37-50.
- Hill, R.E., Favor, J., Hogan, B.L., Ton, C.C., Saunders, G.F., Hanson, I.M., Prosser, J., Jordan, T., Hastie, N.D., and van Heyningen, V. (1992). Mouse

Small eye results from mutations in a paired-like homeobox-containing gene. *Nature* 355, 750.

Hogan, B.L., Horsburgh, G., Cohen, J., Hetherington, C.M., Fisher, G., and Lyon, M.F. (1986). Small eyes (Sey): a homozygous lethal mutation on chromosome 2 which affects the differentiation of both lens and nasal placodes in the mouse. *J Embryol Exp Morphol* 97, 95-110.

Ingham, P.W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* 335, 25-34.

Jacobsson, L., Kronhamn, J., and Rasmuson-Lestander, A. (2009). The *Drosophila* Pax6 paralogs have different functions in head development but can partially substitute for each other. *Mol Genet Genomics*.

Jang, C.C., Chao, J.L., Jones, N., Yao, L.C., Bessarab, D.A., Kuo, Y.M., Jun, S., Desplan, C., Beckendorf, S.K., and Sun, Y.H. (2003). Two Pax genes, eye gone and eyeless, act cooperatively in promoting *Drosophila* eye development. *Development* 130, 2939-2951.

Jones, C., and Moses, K. (2004). Cell-cycle regulation and cell-type specification in the developing *Drosophila* compound eye. *Seminars in Cell & Developmental Biology* 15, 75-81.

Jun, S., Wallen, R.V., Goriely, A., Kalionis, B., and Desplan, C. (1998). Lune/eye gone, a Pax-like protein, uses a partial paired domain and a homeodomain for DNA recognition. *Proc Natl Acad Sci U S A* 95, 13720-13725.

Jym, M. (1995). Spatial regulation of segment polarity gene expression in the anterior terminal region of the *Drosophila* blastoderm embryo. *Mechanisms of Development* 50, 151-161.

Kammermeier, L., Leemans, R., Hirth, F., Flister, S., Wenger, U., Walldorf, U., Gehring, W., and Reichert, H. (2001). Differential expression and function of the *Drosophila* Pax6 genes eyeless and twin of eyeless in embryonic central nervous system development. *Mech Dev* 103, 71-78.

Kenyon, K.L., Ranade, S.S., Curtiss, J., Mlodzik, M., and Pignoni, F. (2003). Coordinating proliferation and tissue specification to promote regional identity in the *Drosophila* head. *Dev Cell* 5, 403-414.

Kenyon, K.L., Yang-Zhou, D., Cai, C.Q., Tran, S., Clouser, C., Decene, G., Ranade, S., and Pignoni, F. (2005). Partner specificity is essential for proper function of the SIX-type homeodomain proteins Sine oculis and Optix during fly eye development. *Developmental Biology* 286, 158-168.

Kronhamn, J., Frei, E., Daube, M., Jiao, R., Shi, Y., Noll, M., and Rasmuson-Lestander, A. (2002). Headless flies produced by mutations in the

paralogous Pax6 genes eyeless and twin of eyeless. *Development* 129, 1015-1026.

Kumar, J.P., and Moses, K. (2001a). EGF receptor and Notch signaling act upstream of Eyeless/Pax6 to control eye specification. *Cell* 104, 687-697.

Kumar, J.P., and Moses, K. (2001b). Eye specification in *Drosophila*: perspectives and implications. *Semin Cell Dev Biol* 12, 469-474.

Lichtneckert, R., and Reichert, H. (2005). Insights into the urbilaterian brain: conserved genetic patterning mechanisms in insect and vertebrate brain development. *Heredity* 94, 465-477.

Mardon, G., Solomon, N.M., and Rubin, G.M. (1994). dachshund encodes a nuclear protein required for normal eye and leg development in *Drosophila*. *Development* 120, 3473-3486.

Martin, P., Carriere, C., Dozier, C., Quatannens, B., Mirabel, M.A., Vandenbunder, B., Stehelin, D., and Saule, S. (1992). Characterization of a paired box- and homeobox-containing quail gene (Pax-QNR) expressed in the neuroretina. *Oncogene* 7, 1721-1728.

Niimi, T., Seimiya, M., Kloter, U., Flister, S., and Gehring, W.J. (1999). Direct regulatory interaction of the eyeless protein with an eye-specific enhancer in the sine oculis gene during eye induction in *Drosophila*. *Development* 126, 2253-2260.

Noll, M. (1993). Evolution and role of Pax genes. *Curr Opin Genet Dev* 3, 595-605.

Pappu, K.S., and Mardon, G. (2004). Genetic control of retinal specification and determination in *Drosophila*. *Int J Dev Biol* 48, 913-924.

Pappu, K.S., Ostrin, E.J., Middlebrooks, B.W., Sili, B.T., Chen, R., Atkins, M.R., Gibbs, R., and Mardon, G. (2005). Dual regulation and redundant function of two eye-specific enhancers of the *Drosophila* retinal determination gene dachshund. *Development* 132, 2895-2905.

Pauli, T., Seimiya, M., Blanco, J., and Gehring, W.J. (2005). Identification of functional sine oculis motifs in the autoregulatory element of its own gene, in the eyeless enhancer and in the signalling gene hedgehog. *Development* 132, 2771-2782.

Pignoni, F., Hu, B., Zavitz, K.H., Xiao, J., Garrity, P.A., and Zipursky, S.L. (1997). The eye-specification proteins So and Eya form a complex and regulate multiple steps in *Drosophila* eye development. *Cell* 91, 881-891.

Plaza, S., Prince, F., Jaeger, J., Kloter, U., Flister, S., Benassayag, C., Cribbs, D., and Gehring, W.J. (2001). Molecular basis for the inhibition of *Drosophila* eye development by Antennapedia. *EMBO J* 20, 802-811.

Punzo, C., Plaza, S., Seimiya, M., Schnupf, P., Kurata, S., Jaeger, J., and Gehring, W.J. (2004). Functional divergence between *eyeless* and *twin of eyeless* in *Drosophila melanogaster*. *Development* *131*, 3943-3953.

Punzo, C., Seimiya, M., Flister, S., Gehring, W.J., and Plaza, S. (2002). Differential interactions of *eyeless* and *twin of eyeless* with the *sine oculis* enhancer. *Development* *129*, 625-634.

Püschel, A.W., Gruss, P., and Westerfield, M. (1992). Sequence and expression pattern of *pax-6* are highly conserved between zebrafish and mice. *Development* *114*, 643-651.

Quiring, R., Walldorf, U., Kloter, U., and Gehring, W. (1994). Homology of the *eyeless* gene of *Drosophila* to the *Small eye* gene in mice and *Aniridia* in humans. *Science* *265*, 785-789.

Ready, D.F., Hanson, T.E., and Benzer, S. (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. *Developmental Biology* *53*, 217-240.

Rodrigues, A.B., and Moses, K. (2004). Growth and specification: fly *Pax6* homologs *eyegone* and *eyeless* have distinct functions. *Bioessays* *26*, 600-603.

Royet, J., and Finkelstein, R. (1995). Pattern formation in *Drosophila* head development: the role of the orthodenticle homeobox gene. *Development* *121*, 3561-3572.

Royet, J., and Finkelstein, R. (1996). hedgehog, wingless and orthodenticle specify adult head development in *Drosophila*. *Development* *122*, 1849-1858.

Royet, J., and Finkelstein, R. (1997). Establishing primordia in the *Drosophila* eye-antennal imaginal disc: the roles of decapentaplegic, wingless and hedgehog. *Development* *124*, 4793-4800.

Rubin, G.M., and Lewis, E.B. (2000). A brief history of *Drosophila*'s contributions to genome research. *Science* *287*, 2216-2218.

Salzer, C.L., and Kumar, J.P. (2009). Position dependent responses to discontinuities in the retinal determination network. *Developmental Biology* *326*, 121-130.

Schedl, A., Ross, A., Lee, M., Engelkamp, D., Rashbass, P., van Heyningen, V., and Hastie, N.D. (1996). Influence of *PAX6* gene dosage on development: overexpression causes severe eye abnormalities. *Cell* *86*, 71-82.

Schmidt-Ott, U., González-Gaitán, M., Jäckle, H., and Technau, G.M. (1994). Number, identity, and sequence of the *Drosophila* head segments as revealed by neural elements and their deletion patterns in mutants. *Proc Natl Acad Sci U S A* *91*, 8363-8367.

- Seimiya, M., and Gehring, W.J. (2000). The *Drosophila* homeobox gene *optix* is capable of inducing ectopic eyes by an eyeless-independent mechanism. *Development* *127*, 1879-1886.
- Shen, W., and Mardon, G. (1997). Ectopic eye development in *Drosophila* induced by directed *dachshund* expression. *Development* *124*, 45-52.
- Singh, A., Kango-Singh, M., and Sun, Y.H. (2002). Eye suppression, a novel function of *teashirt*, requires Wingless signaling. *Development* *129*, 4271-4280.
- Ton, C.C.T., Hirvonen, H., Miwa, H., Weil, M.M., Monaghan, P., Jordan, T., van Heyningen, V., Hastie, N.D., Meijers-Heijboer, H., Drechsler, M., *et al.* (1991). Positional cloning and characterization of a paired box- and homeobox-containing gene from the aniridia region. *Cell* *67*, 1059-1074.
- Treisman, J., Harris, E., and Desplan, C. (1991). The paired box encodes a second DNA-binding domain in the paired homeo domain protein. *Genes Dev* *5*, 594-604.
- Treisman, J.E., and Rubin, G.M. (1995). *wingless* inhibits morphogenetic furrow movement in the *Drosophila* eye disc. *Development* *121*, 3519-3527.
- Walldorf, U., and Gehring, W.J. (1992). *Empty spiracles*, a gap gene containing a homeobox involved in *Drosophila* head development. *EMBO J* *11*, 2247-2259.
- Walther, C., and Gruss, P. (1991). *Pax-6*, a murine paired box gene, is expressed in the developing CNS. *Development* *113*, 1435-1449.
- Wang, L.-H., Chiu, S.-J., and Sun, Y.H. (2008). Temporal switching of regulation and function of eye gene (*eyg*) in *Drosophila* eye development. *Developmental Biology* *321*, 515-527.
- Wawersik, S., and Maas, R.L. (2000). Vertebrate eye development as modeled in *Drosophila*. *Hum Mol Genet* *9*, 917-925.
- Wilson, D.S., Guenther, B., Desplan, C., and Kuriyan, J. (1995). High resolution crystal structure of a paired (Pax) class cooperative homeodomain dimer on DNA. *Cell* *82*, 709-719.
- Wimmer, E.A., Jäckle, H., Pfeifle, C., and Cohen, S.M. (1993). A *Drosophila* homologue of human Sp1 is a head-specific segmentation gene. *Nature* *366*, 690-694.
- Wimmer, E.A., Simpson-Brose, M., Cohen, S.M., Desplan, C., and Jäckle, H. (1995). Trans- and cis-acting requirements for blastodermal expression of the head gap gene *buttonhead*. *Mechanisms of Development* *53*, 235-245.

Yao, J.-G., Weasner, B.M., Wang, L.-H., Jang, C.-C., Weasner, B., Tang, C.-Y., Salzer, C.L., Chen, C.-H., Hay, B., Sun, Y.H., *et al.* (2008). Differential requirements for the Pax6(5a) genes *eyegone* and *twin of eyegone* during eye development in *Drosophila*. *Developmental Biology* *315*, 535-551.

Younossi-Hartenstein, A., Green, P., Liaw, G.J., Rudolph, K., Lengyel, J., and Hartenstein, V. (1997). Control of early neurogenesis of the *Drosophila* brain by the head gap genes *tll*, *otd*, *ems*, and *btd*. *Dev Biol* *182*, 270-283.

Younossi-Hartenstein, A., Tepass, U., and Hartenstein, V. (1993). Embryonic origin of the imaginal discs of the head of *Drosophila melanogaster*. *Roux's Archive of Developmental biology* *203*, 60-73.

Zimmerman, J.E., Bui, Q.T., Liu, H., and Bonini, N.M. (2000). Molecular genetic analysis of *Drosophila* eyes absent mutants reveals an eye enhancer element. *Genetics* *154*, 237-246.