1. **Kumar JP.** Building an ommatidium one cell at a time. Dev Dyn. 2012 Jan;241(1):136-49. doi: 10.1002/dvdy.23707. Department of Biology, Indiana University, Bloomington, Indiana. [jkumar@indiana.edu](mailto:jkumar@indiana.edu). PMID: 22174084

Since the discovery of a single white-eyed male in a population of red eyed flies over 100 years ago (Morgan, 1910), the compound eye of the fruit fly, Drosophila melanogaster, has been a favorite experimental system for identifying genes that regulate various aspects of development. For example, a fair amount of what we know today about enzymatic pathways and vesicular transport is due to the discovery and subsequent characterization of eye color mutants such as white. Likewise, our present day understanding of organogenesis has been aided considerably by studies of mutations, such as eyeless, that either reduce or eliminate the compound eyes. But by far the phenotype that has provided levers into the greatest number of experimental fields has been the humble "rough" eye. The fly eye is composed of several hundred unit-eyes that are also called ommatidia. These unit eyes are packed into a hexagonal array of remarkable precision. The structure of the eye is so precise that it has been compared with that of a crystal (Ready et al., 1976). Even the slightest perturbations to the structure of the ommatidium can be visually detected by light or electron microscopy. The cause for this is two-fold: (1) any defect that affects the hexagonal geometry of a single ommatidium can and will disrupt the positioning of surrounding unit eyes thereby propagating structural flaws and (2) disruptions in genes that govern the development of even a single cell within an ommatidium will affect all unit eyes. In both cases, the effect is the visual magnification of even the smallest imperfection. Studies of rough eye mutants have provided key insights into the areas of cell fate specification, lateral inhibition, signal transduction, transcription factor networks, planar cell polarity, cell proliferation, and programmed cell death just to name a few. This review will attempt to summarize the key steps that are required to assemble each ommatidium. Developmental Dynamics 241:136-149, 2012. © 2011 Wiley Periodicals, Inc. Copyright © 2011 Wiley Periodicals, Inc.


In the Drosophila eye the retinal determination (RD) network controls both tissue specification and cell proliferation. Mutations in network members result in severe reductions in the size of the eye primordium and the transformation of the eye field into head cuticle. The zinc-finger transcription factor Teashirt (Tsh) plays a role in promoting cell proliferation in the anterior most portions of the eye field as well as in inducing ectopic eye formation in forced expression assays. Tiptop (Tio) is a recently discovered paralog of Tsh. It is distributed in an identical pattern to Tsh within the retina and can also promote ectopic eye development. In a previous study we demonstrated that Tio can induce ectopic eye formation in a broader range of cell populations than Tsh and is also a more potent inducer of cell proliferation. Here we have focused on understanding the molecular and biochemical basis that underlies these differences. The two paralogs are structurally similar but differ in one significant aspect: Tsh contains three zinc finger motifs while Tio has four such domains. We used a series of deletion and chimeric proteins to identify the zinc finger domains that are selectively used for either promoting cell
proliferation or inducing eye formation. Our results indicate that for both proteins the second zinc finger is essential to the proper functioning of the protein while the remaining zinc finger domains appear to contribute but are not absolutely required. Interestingly, these domains antagonize each other to balance the overall activity of the protein. This appears to be a novel internal mechanism for regulating the activity of a transcription factor. We also demonstrate that both Tsh and Tio bind to C-terminal Binding Protein (CtBP) and that this interaction is important for promoting both cell proliferation and eye development. And finally we report that the physical interaction that has been described for Tsh and Homothorax (Hth) do not occur through the zinc finger domains. Copyright © 2010 Elsevier Inc. All rights reserved.

3. Kumar JP. My what big eyes you have: how the Drosophila retina grows. Dev Neurobiol. 2011 Dec;71(12):1133-52. doi: 10.1002/dneu.20921. Department of Biology, Indiana University, Bloomington, USA. jkumar@indiana.edu PMCID: PMC3212655; PMID: 21604387

The compound eye of the fruit fly, Drosophila melanogaster, has for decades been used extensively to study a number of critical developmental processes including tissue development, pattern formation, cell fate specification, and planar cell polarity. To a lesser degree it has been used to examine the cell cycle and tissue proliferation. Discovering the mechanisms that balance tissue growth and cell death in developing epithelia has traditionally been the realm of those using the wing disc. However, over the last decade a series of observations has demonstrated that the eye is a suitable and maybe even preferable tissue for studying tissue growth. This review will focus on how growth of the retina is controlled by the genes and pathways that govern the specification of tissue fate, the division of the epithelium into dorsal-ventral compartments, the initiation, and progression of the morphogenetic furrow and the second mitotic wave. Copyright © 2011 Wiley Periodicals, Inc.


The retinal determination (RD) network in Drosophila comprises 14 known nuclear proteins that include DNA-binding proteins, transcriptional coactivators, kinases, and phosphatases. The composition of the network varies considerably throughout the animal kingdom, with the network in several basal insects having fewer members and with vertebrates having potentially significantly higher numbers of RD genes. One important contributing factor for the variation in gene number within the network is gene duplication. For example, 10 members of the RD-network in Drosophila are derived from duplication events. Here we present an analysis of the coding regions of the five pairs of duplicate genes from within the RD network of several different Drosophila species. We demonstrate that there is differential selection across the coding regions of all RD genes.

Additionally, some of the most significant differences in ratios of non-silent-to-silent site substitutions (d(N)/d(S)) between paralog pairs are found within regions that have no ascribed function. Previous
structure/function analyses of several duplicate genes have identified areas within one gene that contain novel activities when compared with its paralog. The evolutionary analysis presented here identifies these same areas in the paralogs as being under high levels of relaxed selection. We suggest that sequence divergence between paralogs and selection signatures can be used as a reasonable predictor of functional changes in rapidly evolving motifs. © 2011 Wiley Periodicals, Inc.


The road to producing an eye begins with the decision to commit a population of cells to adopting an eye tissue fate, the process of retinal determination. Over the past decade and a half, a network of transcription factors has been found to mediate this process in all seeing animals. This retinal determination network is known to regulate not only tissue fate but also cell proliferation, pattern formation, compartment boundary establishment, and even retinal cell specification. The compound eye of the fruit fly, Drosophila melanogaster, has proven to be an excellent experimental system to study the mechanisms by which this network regulates organogenesis and tissue patterning. In fact the founding members of most of the gene families that make up this network were first isolated in Drosophila based on loss-of-function phenotypes that affect the eye. This chapter will highlight the history of discovery of the retinal determination network and will draw attention to the molecular and biochemical mechanisms that underlie our understanding of how the fate of the retina is determined. Copyright © 2010 Elsevier Inc. All rights reserved.


BACKGROUND: The retinal determination (RD) network is an evolutionarily conserved regulatory circuit that governs early events in the development of eyes throughout the animal kingdom. Ectopic expression of many members of this network leads to the transformation of non-retinal epithelia into eye tissue. An often-overlooked observation is that only particular cell-populations within a handful of tissues are capable of having their primary developmental instructions superseded and overruled.

METHODOLOGY/PRELIMINARY FINDINGS: Here we confirm that indeed, only a discrete number of cell populations within the imaginal discs that give rise to the head, antenna, legs, wings and halteres have the cellular plasticity to have their developmental fates altered. In contrast to previous reports, we find that all transformable cell populations do not lie within the TGFbeta or Hedgehog signaling domains. Additionally neither signaling cascade alone is sufficient for non-retinal cell types to be converted into retinal tissue. The transformation "hot spots" that we have identified appear to coincide with several previously defined transdetermination "weak spots", suggesting that ectopic eye formation is less the result of one network overriding the orders of another, as previously thought, but rather is the physical manifestation of redirecting cell populations of enormous cellular plasticity. We also demonstrate that the initiation of eye formation in non-retinal tissues occurs asynchronously compared to that of the
normal eye suggesting that retinal development is not under the control of a global developmental clock.

CONCLUSIONS/SIGNIFICANCE: We conclude that the subregions of non-retinal tissues that are capable of supporting eye formation represent specialized cell-populations that have a different level of plasticity than other cells within these tissues and may be the founder cells of each tissue.


Members of the Eyes absent (Eya) protein family play important roles in tissue specification and patterning by serving as both transcriptional activators and protein tyrosine phosphatases. These activities are often carried out in the context of complexes containing members of the Six and/or Dach families of DNA binding proteins. eyes absent, the founding member of the Eya family is expressed dynamically within several embryonic, larval, and adult tissues of the fruit fly, Drosophila melanogaster. Loss-of-function mutations are known to result in disruptions of the embryonic head and central nervous system as well as the adult brain and visual system, including the compound eyes. In an effort to understand how eya is regulated during development, we have carried out a genetic screen designed to identify genes that lie upstream of eya and govern its expression. We have identified a large number of putative regulators, including members of several signaling pathways. Of particular interest is the identification of both yan/anterior open and pointed, two members of the EGF Receptor (EGFR) signaling cascade. The EGFR pathway is known to regulate the activity of Eya through phosphorylation via MAPK. Our findings suggest that this pathway is also used to influence eya transcriptional levels. Together these mechanisms provide a route for greater precision in regulating a factor that is critical for the formation of a wide range of diverse tissues.


Alphaviruses are RNA viruses transmitted between vertebrate hosts by arthropod vectors, primarily mosquitoes. How arthropods counteract alphaviruses or viruses per se is not very well understood. Drosophila melanogaster is a powerful model system for studying innate immunity against bacterial and fungal infections. In this study we report the use of a novel system to analyze replication of Sindbis virus (type species of the alphavirus genus) RNA following expression of a Sindbis virus replicon RNA from the fly genome. We demonstrate deficits in the immune deficiency (Imd) pathway enhance viral replication while mutations in the Toll pathway fail to affect replication. Similar results were observed with intrathoracic injections of whole virus and confirmed in cultured mosquito cells. These findings show that the Imd pathway mediates an antiviral response to Sindbis virus replication. To our knowledge, this is the first demonstration of an antiviral role for the Imd pathway in insects.

The Sine Oculis Homeobox (SIX) proteins play critical roles in organogenesis and are defined by the presence of two evolutionarily conserved functional motifs: a homeobox DNA binding domain and the SIX protein-protein interaction domain. Members of this transcription factor family can be divided into three subgroups: Six1/2, Six4/5, and Six3/6. This partitioning is based mainly on protein sequence similarity and genomic architecture, and not on specificities of DNA binding or binding partners. In fact, it is well demonstrated that members of the different subgroups can bind to and activate common transcriptional targets as well as form biochemical complexes with communal binding partners. Here we report that the C-terminal segment, which is not conserved across different SIX subfamilies, may serve to functionally distinguish individual SIX proteins. In particular, we have dissected the C-terminal region of Optix, the Drosophila ortholog of mammalian Six3/6, and identified three regions that distinguish Optix from Sine Oculis, the fly homolog of Six1/2. Two of these regions have been preserved in all Six3/6 family members while the third section is present only within Optix proteins in the Drosophilids. The activities of these regions are required, in unison, for Optix function. We suggest that biochemical/functional differences between members of large protein families as well as proteins encoded by duplicate genes can, in part, be attributed to the activities of nonconserved segments. Finally, we demonstrate that a subset of vertebrate SIX proteins has retained the ability to function during normal fly eye development but have lost the ability to induce the formation of ectopic eyes.


Pax genes encode DNA binding proteins that play pivotal roles in the determination of complex tissues. Members of one subclass, Pax6, function as selector genes and play key roles in the retinal development of all seeing animals. Mutations within the Pax6 homologs including fly eyeless, mouse Small eye and human Pax6 lead to severe retinal defects in their respective systems. In Drosophila eyeless and twin of eyeless, play non-redundant roles in the developing retina. One particularly interesting characteristic of these genes is that, although expression of either gene can induce ectopic eye formation in non-retinal tissues, there are differences in the location and frequencies at which the eyes develop. eyeless induces much larger ectopic eyes, at higher frequencies, and in a broader range of tissues than twin of eyeless. In this report we describe a series of experiments conducted in both yeast and flies that has identified protein modules that are responsible for the differences in tissue transformation. These domains appear to contain transcriptional activator and repressor activity of distinct strengths. We propose a model in which the selective presence of these activities and their relative strengths accounts, in part, for the disparity to which ectopic eyes are induced in response to the forced expression of eyeless and twin of eyeless. The identification of both transcriptional activator and repressor activity within the Pax6 protein furthers our understanding of how this gene family regulates tissue determination.
11. Datta RR, Lurye JM, Kumar JP. Restriction of ectopic eye formation by Drosophila teashirt and tiptop to the developing antenna. Dev Dyn. 2009 Sep;238(9):2202-10. Department of Biology, Indiana University, Bloomington, Indiana 47405, USA. PMCID: PMC2733933; PMID: 19347955

In Drosophila, the retinal determination network comprises a set of nuclear factors whose loss-of-function phenotypes often include the complete or near total elimination of the developing eye. These genes also share the ability of being able to induce ectopic eye formation when forcibly expressed in nonretinal tissues such as the antennae, legs, halteres, wings, and genitals. However, it appears that the ability to redirect and transform tissue fates is limited; not all tissues and cell populations can be forced into adopting an eye fate. In this report, we demonstrate that ectopic eye formation by teashirt and its paralog tiptop, a potential new eye specification gene, is restricted to the developing antennae. Of interest, tiptop appears to be a more effective inducer of retinal formation than teashirt. A genetic screen for interacting proteins failed to identify paralog-specific relationships suggesting that the differences between these two genes may be attributed instead to structural differences between the duplicates. We also demonstrate that in addition to being expressed in coincident patterns within the developing eye, both paralogs are transcribed at very similar levels. 2009 Wiley-Liss, Inc.


The development of any cell and/or tissue is dependent upon interconnections between several signaling pathways and myriad transcription factors. It is becoming more apparent that these inputs are best studied, not as individual components, but rather as elements of a gene regulatory network. Over the last decade several networks governing the specification of single cells, individual organs and entire stages of development have been described. The current incarnations of these networks are the products of the continual addition of newly discovered genetic, molecular and biochemical interactions. However, as currently envisaged, network diagrams may not sufficiently describe the spatial and temporal dynamics that underlie developmental processes. We have conducted a developmental analysis of a sub circuit of the Drosophila retinal determination network. This sub circuit is comprised of three genes, two (sine oculis and dachshund) of which code for DNA binding proteins and one (eyes absent) that encodes a transcriptional co-activator. We demonstrate here that the nature of the regulatory relationships that exist between these three genes changes as retinal development progresses. We also demonstrate that the response of the tissue to the loss of any of these three RD genes is dependent upon the position of the mutant cells within the eye field. Depending upon its location, mutant tissue will either overproliferate itself or will signal to surrounding cells instructing them to propagate and compensate for the eventual loss through apoptosis of the mutant clone. Taken together these results suggest that the complexities of development are best appreciated when spatial and temporal information is incorporated when describing gene regulatory networks.
The developing eye of the fruit fly, Drosophila melanogaster, has become a premier model system for studying the genetic and molecular mechanisms that govern tissue determination. Over the last fifteen years a regulatory circuit consisting of the members of the Pax, Six, Eya and Dach gene families has been identified and shown to govern the specification of a wide range of tissues including the retina of both insects and mammals. These genes are not organized in a simple developmental pathway or cascade in which there is a unidirectional flow of information. Rather, there are multiple feedback loops built into the system rendering its appearance and functionality more in line with the workings of a network. In this review I will attempt to describe the genetic, molecular and biochemical interactions that govern the specification of the Drosophila compound eye. In particular, the primary focus will be on the interactions that have been experimentally verified at the molecular and biochemical levels. During the course of this description I will also attempt to place each discovery in its own historical context. While a number of signaling pathways play significant roles in early eye development this review will focus on the network of nuclear factors that promote retinal determination.

In eye development the tasks of tissue specification and cell proliferation are regulated, in part, by the Pax6 and Pax6(5a) proteins respectively. In vertebrates, Pax6(5a) is generated as an alternately spliced isoform of Pax6. This stands in contrast to the fruit fly, Drosophila melanogaster, which has two Pax6(5a) homologs that are encoded by the eyegone and twin of eyegone genes. In this report we set out to determine the respective contributions that each gene makes to the development of the fly retina. Here we demonstrate that both eyg and toe encode transcriptional repressors, are expressed in identical patterns but at significantly different levels. We further show, through a molecular dissection of both proteins, that Eyg makes differential use of several domains when compared to Toe and that the number of repressor domains also differs between the two Pax6(5a) homologs. We predict that these results will have implications for elucidating the functional differences between closely related members of other Pax subclasses.

The initiation of eye formation in all seeing animals is controlled by a group of selector genes that together forms the retinal determination cascade. In Drosophila, mice and humans, loss-of-function mutations lead to defects in eye and/or head development. While ectopic expression of these genes is sufficient to direct non-retinal tissues towards an eye fate, the ability of each gene to initiate eye formation is neither unlimited nor equal. A particularly enigmatic observation has been that one member of the cascade, sine oculis (so), which is a member of the SIX family of homeobox transcription factors, is unable to initiate eye development in non-retinal tissues. It is in contrast to every other retinal determination gene including optix, another Six family member, which can induce eye formation when expressed on its own. Here we demonstrate that, in contrast to published reports, expression of so on its own is sufficient to induce eye development within non-retinal tissues. We have extended results from prior reports on binding partner selectivity and DNA binding sites by conducting a structure/function analysis of the SO and OPTIX proteins. Here we demonstrate that the SIX domains and C-terminal portions of the SO and OPTIX proteins are required for functional specificity of SIX class transcription factors while the homeodomain of these proteins are interchangeable. Taken together, these results shed new light on the role that so plays in eye specification.


The retinal determination gene dachshund is distantly related to the family of Ski/Sno proto-oncogenes and influences the development of a wide range of tissues including the embryonic head, optic lobes, brain, central nervous system as well as the post-embryonic leg, wing, genital and eye-antennal discs.

We were interested in the regulatory mechanisms that control the dynamic expression pattern of dachshund and in this report we set out to ascertain how the transcription of dachshund is modulated in the embryonic head and developing eye-antennal imaginal disc. We demonstrate that the TGFbeta signaling cascade, the transcription factor zerknullt and several other patterning genes prevent dachshund from being expressed inappropriately within the embryonic head. Additionally, we show that several members of the eye specification cascade influence the transcription of dachshund during normal and ectopic eye development. Our results suggest that dachshund is regulated by a complex combinatorial code of transcription factors and signaling pathways. Unraveling this code may lead to an understanding of how dachshund regulates the development of many diverse tissue types including the eye.

Drosophila CREB-binding protein (dCBP) is a very large multidomain protein, which belongs to the CBP/p300 family of proteins that were first identified by their ability to bind the CREB transcription factor and the adenoviral protein E1. Since then CBP has been shown to bind to >100 additional proteins and functions in a multitude of different developmental contexts. Among other activities, CBP is known to influence development by remodeling chromatin, by serving as a transcriptional coactivator, and by interacting with terminal members of several signaling transduction cascades. Reductions in CBP activity are the underlying cause of Rubinstein-Taybi syndrome, which is, in part, characterized by several eye defects, including strabismus, cataracts, juvenile glaucoma, and coloboma of the eyelid, iris, and lens. Development of the Drosophila melanogaster compound eye is also inhibited in flies that are mutant for CBP. However, the vast array of putative protein interactions and the wide-ranging roles played by CBP within a single tissue such as the retina can often complicate the analysis of CBP loss-of-function mutants. Through a series of genetic screens we have identified several genes that could either serve as downstream transcriptional targets or encode for potential CBP-binding partners and whose association with eye development has hitherto been unknown. The identification of these new components may provide new insight into the roles that CBP plays in retinal development. Of particular interest is the identification that the CREB transcription factor appears to function with CBP at multiple stages of retinal development.


The development of the compound eye of Drosophila is controlled, in part, by the concerted actions of several nuclear proteins that form an intricate regulatory system. One member of this network is sine oculis (so), the founding member of the Six gene family. Mutations within so affect the entire visual system, including the compound eye. The vertebrate homologs Six3 and Six6 also appear to play crucial roles in retinal formation. Mutations in Six3 inhibit retinal formation in chickens and fish, whereas those in Six6 are the underlying cause of bilateral anophthalmia in humans. Together, these phenotypes suggest a conserved role for the Six genes in eye development. In this report, we describe the effects of a dominant-negative mutation of sine oculis on the development of the compound eye of Drosophila. The mutation resides within the Six domain and may have implications for eye development and disease. Copyright (c) 2005 Wiley-Liss, Inc.

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During the development of the compound eye of Drosophila several signaling pathways exert both positive and inhibitory influences upon an array of nuclear transcription factors to produce a near-perfect lattice of unit eyes or ommatidia. Individual cells within the eye are exposed to many extracellular signals, express multiple surface receptors, and make use of a large complement of cell-subtype-specific DNA-binding transcription factors. Despite this enormous complexity, each cell will make the correct developmental choice and adopt the appropriate cell fate. How this process is managed remains a poorly understood paradigm. Members of the CREB binding protein (CBP)/p300 family have been shown to influence development by (1) acting as bridging molecules between the basal transcriptional machinery and specific DNA-binding transcription factors, (2) physically interacting with terminal members of signaling cascades, (3) acting as transcriptional coactivators of downstream target genes, and (4) playing a key role in chromatin remodeling. In a screen for new genes involved in eye development we have identified the Drosophila homolog of CBP as a key player in both eye specification and cell fate determination. We have used a variety of approaches to define the role of CBP in eye development on a cell-by-cell basis.