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Position dependent responses to discontinuities in the retinal determination network

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ABSTRACT

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.

Introduction

The last decade has played witness to the revelation that the specification of tissues and organs, are regulated, not by simple linear cascades, rather by complicated interconnected gene regulatory networks (GRNs). The influence of such networks can be limited to a single context or can extend to multiple developing tissues. Such is the case for the retinal determination (RD) network, which, in addition to the eye, regulates the fate of a number of tissues in both insect and vertebrate systems. First identified in flies, this network also controls the development of learning and memory centers of the brain, several mesodermal derivatives, the gonads and select cells within the central nervous system (Bai and Montell, 2002; Bonini et al., 1998; Callaerts et al., 2001; Chang et al., 2003; Fabrizio et al., 2003; Kammermeier et al., 2001; Kurusu et al., 2000; Mardon et al., 1994; Niimi et al., 1999; Noveen et al., 2000). In addition to its role in vertebrate eye development, the RD network regulates ear, nose, kidney and muscle specification (Brodbeck and Englert, 2004; Gong et al., 2007; Hammond et al., 1998; Hanson, 2001; Heanue et al., 1999; Kalatzis et al., 1998; Laclef et al., 2003; Relaix and Buckingham, 1999; Simpson and Price, 2002; Xu et al., 2003). Over the years members of seven gene families have been identified to function within the RD network. In *Drosophila* these include the Pax6 genes *eyeless* (*ey*) and *twin of eyeless* (*toy*), the Pax6(5a) genes *eyegone* (*eyg*) and *twin of eyegone* (*toe*), the Six family members *sine oculis* (*so*) and *optix*, the founding member of the Eya family of transcriptional co-activators *eyes absent* (*eya*), a distant relative of the Ski/Sno family of proto-oncogenes *dachshund* (*dac*), the Meis1 homolog *homothorax* (*hth*) and the zinc finger transcription factor *teashirt* (*tsh*) (reviewed in (Kumar and Moses, 2001b; Treisman, 1999; Treisman and Heberlein, 1998; Weasner et al., 2004).

The evidence that prompted the placement of these genes into a functional network is principally drawn from loss-of-function mutant phenotypes (Bonini et al., 1993; Cheyette et al., 1994; Jang et al., 2003; Mardon et al., 1994; Quiring et al., 1994; Serikaku and O'Tousa, 1994), overlapping expression patterns (Bessa et al., 2002), direct transcriptional activation of one gene by another (Czerny et al., 1999; Niimi et al., 1999; Ostrin et al., 2006; Pauli et al., 2005), protein–protein interactions amongst selected network members (Chen et al., 1997; Pignoni et al., 1997) and the unique ability of these genes to induce ectopic eyes in non-retinal tissues (Bonini et al., 1997; Czerny et al., 1999; Halder et al., 1995; Pan and Rubin, 1998; Seimiya and Gehring, 2000; Shen and Mardon, 1997; Weasner et al., 2007). As additional experimental evidence is gathered, new positive or inhibitory arrows are added resulting in a network with ever increasing complexity. Similar GRNs with equal or greater complexity have been identified in a number of systems including the fly wing and ventral furrow

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(Aracena et al., 2006; Guss et al., 2001); mouse stem cell, B lymphocyte and brain (Li et al., 2007; Medina et al., 2004; Wang et al., 2007; Zhou et al., 2007); *Xenopus* mesoendoderm (Loose and Patient, 2004); vertebrate neural crest (Sauka-Spengler and Bronner-Fraser, 2008a; Sauka-Spengler and Bronner-Fraser, 2008b; Sauka-Spengler et al., 2007); *Arabidopsis* flower development (Espinosa-Soto et al., 2004) and sea urchin embryogenesis (Davidson et al., 2002; Oliveri et al., 2002; Oliveri and Davidson, 2004a; Oliveri and Davidson, 2004b) just to name a few. However, as is the case with any complex system, no single regulatory model can fully describe all of the spatial and temporal events that occur during development (Flores et al., 2000) to produce the final adult tissue.

Eye specification in *Drosophila* begins during embryogenesis when a small group of cells are set aside to give rise to the future compound eye (Cohen, 1993). Upon emerging as a larva, these cells become organized into a monolayer epithelium called the eye-antennal imaginal disc. During the first two larval instars the eye disc undergoes massive proliferation to generate the large numbers of cells that are required to produce the approximately 800 unit eyes or ommatidia that comprise the adult compound eye. At the start of the third and final instar, pattern formation is initiated at the posterior margin of the epithelium. The wave of morphogenesis can be visualized by a dorso-ventral groove in the epithelium referred to as the morphogenetic furrow (Ready et al., 1976). As the furrow passes, the pool of undifferentiated cells are organized into periodic clusters of developing ommatidia (Ready et al., 1976; Wolff and Ready, 1991). Within each cluster are approximately twenty cells that adopt either photoreceptor or non-neuronal accessory cell fates (Cagan and Ready, 1989; Tomlinson and Ready, 1987a; Tomlinson and Ready, 1987b). These decisions involve complex, stereotyped patterns of gene expression (Dickson and Hafen, 1993; Doroquez and Rebay, 2006; Flores et al., 2000; Kumar and Moses, 1997; Nagaraj and Banerjee, 2007; Voas and Rebay, 2004). Ultimately, the several hundred ommatidia are organized into a precise hexagonal array characteristic of the adult retina.

In the developing fly retina *ey* is one of the first RD genes to be expressed. Along with *toy*, *ey* directly activates the transcription of several downstream targets including itself and three other network genes: *so*, *optix* and *eya* (Halder et al., 1998; Niimi et al., 1999; Ostrin et al., 2006). So and Eya proteins form a composite transcription factor with So contributing a DNA binding domain and Eya providing an activation domain (Pignoni et al., 1997). The So–Eya complex, in turn, activates a number of target genes that play crucial roles in cell proliferation (*string*, (Jemc and Rebay, 2007), pattern formation (*hedgehog*, (Pauli et al., 2005) and cell fate specification (*lozenge*, (Yan et al., 2003). Additionally, So–Eya feeds back to regulate the transcription of the upstream gene *ey* (Pauli et al., 2005) and the downstream target *dac* (Pappu et al., 2005). It is this last interaction that is the central focus of this report, as it highlights an instance in which the totality of experimental evidence is not represented by the most current network models.

Consistent with their roles as obligate partners, So and Eya proteins are distributed in completely overlapping expression patterns in the developing eye. Both are expressed in a swathe of undifferentiated cells ahead of the advancing morphogenetic furrow and in all cells posterior to the furrow (Bonini et al., 1993; Cheyette et al., 1994; Serikaku and O'Tousa, 1994). Dac protein distribution ahead of the furrow overlaps that of So and Eya. However, posterior to the furrow *dac* expression is maintained for approximately eight rows where it is restricted to only a subset of photoreceptors and then quickly tapers off (Mardon et al., 1994). Two enhancers responsible for the activation of *dac* expression in the retina are under the partial control of both *so* and *eya* (Pappu et al., 2005). As the So–Eya complex is still present and functioning in the more posterior cells it is intriguing that *dac* expression ceases. The seminal experiments that established the regulatory relationships among the RD genes were based in large measure on immunohistochemical assays completed in entirely mutant eye discs in which a furrow failed to initiate (Anderson et al., 2006; Chen et al., 1997; Halder et al., 1998; Pappu et al., 2005) and in ectopic eye assays in which the distribution of RD proteins were measured in response forced expression of either individual or combinations of genes (Bonini et al., 1997; Chen et al., 1999; Czerny et al., 1999; Halder et al., 1995; Shen and Mardon, 1997; Weasner et al., 2007). These experiments have been critical to our understanding of the regulatory interactions that take place during nascent phases of eye development and within the anterior compartment of the developing retina. Several regulatory relationships, first established genetically, have been supported by evidence of protein–protein interactions and direct transcriptional regulatory relationships (Chen et al., 1999; Czerny et al., 1999; Michaut et al., 2003; Niimi et al., 1999; Ostrin et al., 2006; Pauli et al., 2005; Pignoni et al., 1997).

A distinct disadvantage to this historical approach is that interactions taking place along the margins, at the D/V and A/P boundaries, and in cells posterior to the furrow cannot be assessed and thus have largely been neglected. This is particularly true of *so*, *eya* and *dac*, which are the only three RD genes to be expressed posterior the furrow (Bonini et al., 1993; Cheyette et al., 1994; Mardon et al., 1994; Serikaku and O'Tousa, 1994). All three genes are required for furrow initiation and the So–Eya complex is required in the R1, R6 and R7 photoreceptors (Mardon et al., 1994; Pignoni et al., 1997). However it is unclear if the regulatory relationships existing among the three genes in anterior regions of the eye also exist along the posterior regions where pattern formation initiates and in differentiating photoreceptor neurons. In order to verify existing interactions or identify new regulatory relationships among *so*, *eya* and *dac*, we generated randomly distributed retinal mosaic clones for each gene and determined the effect that loss of each gene had on the expression of the other two factors.

Here we show that the response of the eye to discontinuities in the retinal determination network is not static across the eye field but rather is dynamic and position dependent. In particular we demonstrate that, unlike regions anterior the furrow, removal of *so* and *eya* in posterior positions of the eye lead to an attempt by these cells to reinitiate the retinal determination program by expressing RD genes that are normally found exclusively in the anterior compartment. This attempt fails and is then followed by cell suicide via programmed cell death but not before the *so* and *eya* mutant cells non-autonomously signal through the Notch pathway to adjacent undifferentiated cells instructing them to compensate for their loss by activating *dac* expression and proliferating. These surrounding cells, which are not competent to properly execute the RD program neither adopt a retinal fate nor die, therefore they assume a default head cuticle fate. We also demonstrate that the loss of either *so* or *eya* at the margins of the eye epithelium results in a different developmental path. In these cases, the mutant cells themselves will autonomously overproliferate thereby bypassing any requirement for communication with adjacent cell populations. Consistent with this, the adjacent undifferentiated cells do not activate Notch signaling, express *dac* or proliferate. The conclusion that we draw from these observations is that the gene regulatory networks governing early specification and patterning decisions are not static sets of connections but rather are temporally and spatially dynamic.

Materials and methods

Fly stocks

The following stocks were used to generate retinal mosaic clones: *w; FRT40A dac^{E462}* (gift from Graeme Mardon), *w; FRT42D so^{3}* (gift from Francesca Pignoni), *w; FRT42D eya^{2}*, and *w;; FRT82B gro^{E48}* (gift from Janice Fischer) with the following FRT lines: *w; FRT40A Ubi-GFP*, *w; FRT 40A Pw^{+}*, *w; FRT42D Ubi-GFP*, *w; FRT42D Pw^{+}*, *w;; FRT82B Ubi-GFP RpS3*