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FlyBook

The early history of the eye-antennal disc of *Drosophila melanogaster*

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Abstract

A pair of eye-antennal imaginal discs give rise to nearly all external structures of the adult *Drosophila* head including the compound eyes, ocelli, antennae, maxillary palps, head epidermis, and bristles. In the earliest days of *Drosophila* research, investigators would examine thousands of adult flies in search of viable mutants whose appearance deviated from the norm. The compound eyes are dispensable for viability and perturbations to their structure are easy to detect. As such, the adult compound eye and the developing eye-antennal disc emerged as focal points for studies of genetics and developmental biology. Since few tools were available at the time, early researchers put an enormous amount of thought into models that would explain their experimental observations—many of these hypotheses remain to be tested. However, these "ancient" studies have been lost to time and are no longer read or incorporated into today's literature despite the abundance of field-defining discoveries that are contained therein. In this FlyBook chapter, I will bring these forgotten classics together and draw connections between them and modern studies of tissue specification and patterning. In doing so, I hope to bring a larger appreciation of the contributions that the eye-antennal disc has made to our understanding of development as well as draw the readers' attention to the earliest studies of this important imaginal disc. Armed with the today's toolkit of sophisticated genetic and molecular methods and using the old papers as a guide, we can use the eye-antennal disc to unravel the mysteries of development.

Keywords: Drosophila; eye-antennal disc; FlyBook

Historical note

Over the last 120 years, the imaginal discs of the fruit fly, *Drosophila melanogaster*, have been used to gain critical insights into the mechanisms underlying important developmental events such as organ specification, axis formation, tissue patterning, and growth. This has been possible in part because an enormous assortment of genetic, molecular, and microscopy tools have been developed or adapted by the fly community for use in *Drosophila* research. For much of its history, insights into the regulatory networks that control development have come from a painstaking analysis of individual genes and mutant phenotypes—the "one gene at a time" approach. However, in recent years, dramatic advances in the field of genome biology have accelerated efforts to uncover the molecular mechanisms that underlie development. It began with the sequencing of the fly genome, which revealed the full array of genes that control the life of the fly. From there, an array of technologies has emerged to provide insights into not just one gene but rather the entire genome. We can now determine the 3D organization of the genome from any tissue, the pattern of transcription factor binding and epigenetic marks across said genome, as well as the expression profile of any tissue or cell type. At the same time, an explosion in available genetic tools has allowed researchers to fully exploit the information that has been gained from high-throughput genomic methods. For example, any gene can now be either over or ectopically expressed within thousands of distinct temporal and spatial patterns using binary systems such as UAS/GAL4 and LexA/LexAop. Likewise, libraries of RNA interference (RNAi) targeting lines and CRISPR-based genome editing tools allow for virtually any gene to be either knocked down or removed. The effects that gene loss has on development can be analyzed in exquisite detail by ever growing numbers of cell markers, protein traps, transcriptional reporters, lineage markers, and clonal analysis tools. And lastly, groundbreaking advances in light microscopy have made it possible to watch tissues and even individual cells develop in real time. All of these technological advances have allowed us to obtain a remarkable view of development.

Although none of these technologies were available to early *Drosophila* researchers, much of our current understanding of development is predicated on the early precloning era studies of the fly. Armed only with basic histology, Mendelian genetics, gynandromorphs, tissue transplantation, X-ray mutagenesis, and rudimentary tools to generate marked mosaic clones, early researchers made seminal contributions to our understanding of tissue specification, pattern formation, growth control, organizing centers, axis formation, tissue induction, cell–cell communication, planar cell polarity, and signal transduction. The developing compound eye was a particularly attractive tissue to study these topics since even mild mutations have visible, outsized, and profound effects. This is due, in part, to the structure of the compound eye itself. It is comprised of nearly 750 identical unit eyes called ommatidia so a mutation that affects one

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ommatidium affects them all—thus its effect is amplified several hundred times across the entire eye field. Likewise, the ommatidia are interlocked into a hexagonal array, therefore a distortion that begins in one portion of the eye field is propagated throughout the entire retinal array much like the dropping of a stone sends ripples through a pond. The "roughening" of the eye surface, which results from patterning defects, is extremely easy to detect even for the most untrained eye. Thus, it was natural for early researchers to focus on the adult compound eye and the developing eye-antennal disc from which it is derived. The early history of the eye-antennal disc is the subject of this Flybook chapter.

Structure of the eye-antennal disc and adult head

The best and most detailed anatomical descriptions of the adult *Drosophila* head were first published by G. F. Ferris in 1950 and later by Peter Bryant in 1978. The most prominent features are the compound eyes and ocelli which make up the visual system, the antennae and maxillary palps which comprise the olfactory system, the head epidermis, and a myriad of bristle types (Fig. 1a–c) (Ferris 1950; Bryant 1978). Since many mutants identified by early *Drosophila* researchers affected the pigmentation and/or structure of the eye, it became an important tissue for studies of genetics and development (Morgan 1910; Tice 1914; Hoge 1915; Morgan and Bridges 1916; Lancefield 1918; Richards 1918; Bridges and Morgan 1919; Clausen 1924; Mohr 1924). For example, as each new mutant was isolated, considerable effort was placed in assigning each gene a location on 1 of the 4 chromosomes and determining its position relative to other genes. These experiments were instrumental in developing a more sophisticated understanding of Mendel's laws of inheritance. Likewise, early studies of eye development provided key insights into tissue fate specification, pattern formation, and growth.

The first depiction of the eye-antennal disc comes from August Weismann's monograph on postembryonic structures of Diptera. The camera lucida drawings within his book contained all of the major structural features of the disc including what would be later identified as the morphogenetic furrow (Weismann 1864). The disc was initially referred to as a monolayer epithelium but serial sectioning of the imaginal disc, several decades later, showed it to be a sac-like structure that consists of 3 distinct cell layers—a columnar pseudostratified epithelium called the disc proper (DP); an overlying sheet of squamous cells called the peripodial epithelium (PE); and a strip of cuboidal cells called the margin (M). The DP and PE layers are of the same size and shape and lie closely juxtaposed to each other. The 2 epithelial layers are joined together along their edges by the M cells (Krafka 1924; Chen 1929; Pilkington 1942). These 3 cell layers enclose a thin lumen through which signaling molecules can travel. As such, the eye-antennal disc resembles a closed pillowcase (Fig. 2a–c). Subsequent studies of other imaginal discs showed that they all share a similar architecture (Auerbach 1936).

Origin of the adult head

For nearly a century before *Drosophila* emerged as an experimental model system, researchers had been trying to understand the organizational relationship between the embryo and the adult. Anatomical studies of both developing and adult insects had led to the view that the metameric segment was a fundamental unit of cellular determination and differentiation. In other words, the development of each adult segment and all structures contained therein could be traced back to a single embryonic segment. In general, studies of *Drosophila* development using gynandromorphs, mitotic clones, and homeotic mutants appeared to confirm this view (Steiner 1976; Lawrence and Morata 1977; Bryant 1978; Gehring 1978; Lewis 1978; Lawrence 1981). However, there were 2 adult structures that seemed to defy the 1 embryonic segment, 1 adult segment rule. The first is the adult genitalia, which along with the genital imaginal disc, was shown to be comprised of cells from 2 different embryonic segments—abdominal segments 8 and 9 (Gleichauf 1936; Ferris 1950; Dubendorfer 1971; Emmert 1972; Nothiger *et al.* 1977; Schupbach *et al.* 1978; Epper and Nothiger 1982). The second structure to defy the 1 embryonic segment—one adult segment rule is the adult head and its precursor, the eye-antennal disc.

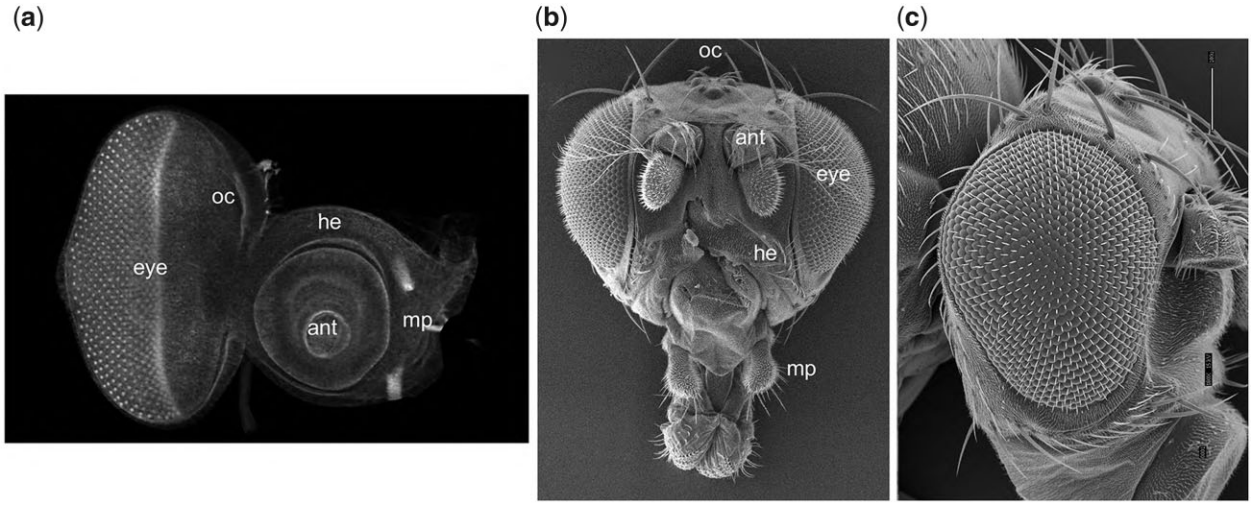

Fig. 1. Fate map of the eye-antennal disc and adult head. Two eye-antennal discs fuse together during pupal development and comprise most of the external structures of the adult head. a) The eye-antennal disc is divided into several individual neighborhoods. These domains give rise to the compound eye (eye), the ocelli (oc), the antenna (ant), the maxillary palps (mp), and all surrounding head epidermis (he). b) The structures that develop within the eye-antennal disc are mapped onto the right side of the adult head. c) A view of the right eye of the compound eye. It contains approximately 750 ommatidia that are organized into 32–34 vertical columns.

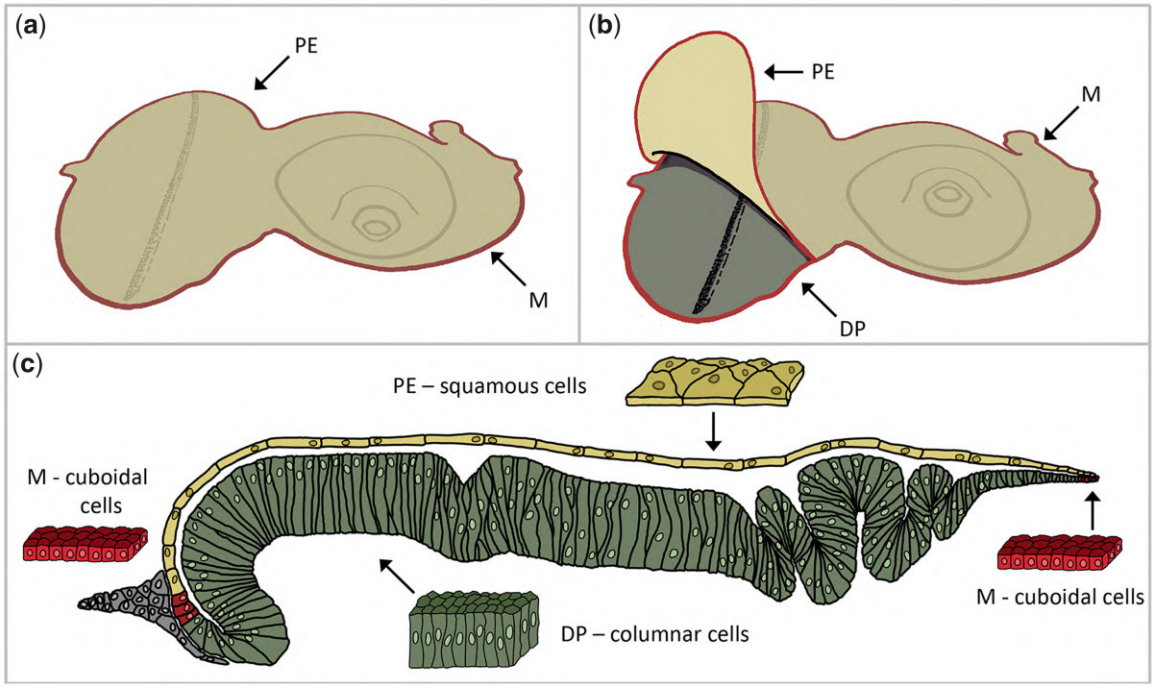

Fig. 2. Structure of the eye-antennal imaginal discs. a–c) The eye-antennal disc is comprised of 3 distinct cell layers. The DP is a pseudo-stratified epithelium consisting of columnar shaped cells. It is covered by a similar sized sheet of squamous cells called the PE. These cells layers are joined together along their edges by a thin layer of cuboidal cells called the margin (M).

Anatomical analyses of the embryonic and adult *Drosophila* head indicated that the latter is comprised of cells from 6 different segments of the former (Crampton 1942; Ferris 1950). This is in strong agreement with what had been described for the general structure of the insect head (Snodgrass 1935). The fly head is derived from 3 pairs of imaginal discs: the eye-antennal disc, the labial disc, and the clypeo-labral disc. The latter 2 discs are derived from distinct embryonic head segments and give rise to distinct portions of the proboscis (Wildermuth and Hadorn 1965; Gehring and Seippel 1967; Wildermuth 1968; Kumar *et al.* 1979). The eye-antennal disc gives rise to the remainder of the head and based on anatomical considerations, was itself thought to come from several separate embryonic cell populations. At the time, the only available paths to directly test this model were to either (1) build a fate map of the blastoderm embryo or (2) see if mitotic clones that were induced during embryogenesis would respect predicted segmental boundaries within the adult head.

Fate maps of the early embryo could be generated by examining sexually mosaic adult gynandromorphs. Such animals are mosaics of XO (male) and XX (female) cells and result from the random loss of X-chromosomes during the earliest stages of embryogenesis (Morgan and Bridges 1919). Naturally occurring gynandromorphs are exceedingly rare in *Drosophila* stocks but it was discovered early on that unstable chromosome (i.e. ring chromosomes) and X-ray induced nondisjunction can be used to experimentally generate gynandromorphs at such frequencies that detailed analyses were now possible (Morgan and Bridges 1919; Mavor 1924; Sturtevant 1929; Schultz and Catcheside 1937). Within a mosaic adult, both male and female cell types could be distinguished from each other by the use of mutant markers for eye color (i.e. *white*), body color (i.e. *yellow*), and/or bristle structure (i.e. *forked*). Adult heads were found to be mosaic for these markers indicating that the head (and the eye-antennal disc by extension) were derived from multiple embryonic cell populations. However, mosaic heads were recovered at very low frequencies, which suggested that the precursors lie in close proximity to each other within the embryo. This was interpreted to mean that the precursor cells for the adult head resided within a single embryonic segment rather than the 6 that were predicted by anatomical studies (Garcia-Bellido and Merriam 1969; Baker 1978; Morata and Lawrence 1979; Struhl 1981a; Haynie and Bryant 1986).

The other approach to studying the organization of the adult head was to see if the descendants of a single embryonic cell were restricted to a single adult head segment or if they would be incorporated into multiple segments. If the adult head is indeed comprised of multiple segments, then a single clone of marked cells (when induced early in embryogenesis) would be contained within and restricted to a single region of the head. On the other hand, if the data from gynandromorphs were correct, then the marked clone would span multiple regions of the adult head. FLP/FRT-based methods for generating clones and lineage tracing tools are recent inventions and did not exist in the early days of *Drosophila* research. Instead, researchers relied on using X-rays to artificially induce recombination events. If recombination occurred within a somatic cell that was heterozygous for a given mutation, then all descendant cells would be of 2 different genotypes—one population would be wild type and the other would be homozygous for the mutation. As with gynandromorph tissue, the cellular products of mitotic recombination could be distinguished from one another in the adult by differences in eye color, body color, and/or bristle structure.

The eye-antennal disc played a pioneering role in the development of clonal analysis methods as the first X-ray-induced mitotic clones were generated within the compound eye. After embryos heterozygous for the *white (w)* mutation were treated with X-rays, the compound eyes of several surviving adults contained patches or clones of white-colored ommatidia that were

surrounded by the normal red color (Patterson 1929). Mitotic clones using mutations in nondevelopmental genes such as *white* were used to answer many questions about how the eye developed. For example, an analysis of the size and shape of *white mutant* clones was used to determine the rate and direction of growth within the compound eye (Becker 1957). Mitotic clones were also used to show that cell–cell interactions and not fixed cell lineage were used to specify all of the cells of the ommatidium (Ready *et al.* 1976; Lawrence and Green 1979). The technique was then adapted to remove developmental genes from patches of tissues. This turned out to be essential for the study of genes that played crucial roles during embryonic development and for which null mutants died prior to advancing to the larval stages.

In order to identify clones that spanned the remaining tissues of the adult head, X-rays were used to induce clones that were mutant for either body color or bristle morphology. In concordance with the data from gynandromorphs, clones induced early in embryogenesis did not respect the boundaries that would be expected if the adult head (minus the proboscis) were derived from multiple embryonic segments. In other words, it appeared that a single clone could encompass 2 tissues such as the eye and the antenna or the antenna and maxillary palp—all of which were originally thought to be derived from distinct segments of the embryo (Postlethwait and Schneiderman 1971; Wieschaus 1974; Gehring 1978). This pattern was observed even when clones were induced as late as the beginning of the third larval instar stage (Becker 1957; Postlethwait and Schneiderman 1971; Morata and Lawrence 1979).

How could the results from gynandromorphs and mitotic clones be reconciled with anatomical studies? Resolution to this question finally came when molecular markers became available for following the movement of cell populations within the embryonic head. An enhancer trap line that reveals the expression of the *escargot (esg)* gene, showed that cells from the antennal segment, acron, labrum, and the 3 gnathal (labium, mandible, maxillary) segments migrate, coalesce, and form the nascent eye-antennal disc (Jurgens and Hartenstein 1993; Younossi-Hartenstein *et al.* 1993). Furthermore, an analysis of the *labial (lab)*, *Deformed (Dfd)*, and *Sex combs reduced (Scr)* homeotic genes showed that while these genes are expressed in distinct segments of the embryo, loss-of-function mutants all result in the deletion or transformation of structures that are derived from the eye-antennal disc (Merrill *et al.* 1987; Diederich *et al.* 1989, 1991; Mahaffey *et al.* 1989; Pattatucci *et al.* 1991; Pederson *et al.* 1996). Together, these data agree with early anatomical studies of *Drosophila* and clearly demonstrate that the adult head and the eye-antennal disc are derived from multiple embryonic segments.

Origin of the eye-antennal disc

While molecular markers were used to show that development of the eye-antennal disc initiated during embryogenesis, the earliest attempts to trace the origin of the eye-antennal disc did not have the benefit of such reagents and instead had to rely on serial sectioning of embryos and larvae. The first attempts pinpointed the origin of the disc to the second larval instar stage (Krafka 1924; Chen 1929). As histological methods improved, discs were seen in newly hatched larvae (Auerbach 1936; Kaliss 1939). This was in general agreement with the opinion at the time that imaginal discs in other insects originate their development during embryogenesis (Pratt 1897). The origin of the disc was traced to early embryogenesis by experiments in which blastoderm stage embryos were subjected to localized damage with glass needles. The resulting adults lacked specific structures such as the wings, eyes, and legs with the loss of individual structures being dependent upon the location of the puncture. These experiments were interpreted to mean that imaginal discs were both set aside and specified during the blastoderm stage of embryogenesis (Geigy 1931; Howland and Child 1935).

More than half a century would pass until antibodies and enhancer trap lines would be available to trace the embryonic development of the eye-antennal disc. As mentioned above, visualizing the distribution of *esg* transcripts allowed for precursors of the eye-antennal disc to be identified in stage 13 embryos. The nascent eye-antennal disc is visible as a single unit for the first time in stage 16/17 embryos (Jurgens and Hartenstein 1993; Younossi-Hartenstein *et al.* 1993). At this time, the expression of several Paired box (Pax) transcription factors including *eyeless (ey)*, *twin of eyeless (toy)*, *eyegone (eyg)*, and *twin of eyegone (toe)* is activated throughout the disc (Quiring *et al.* 1994; Jones *et al.* 1998; Jun *et al.* 1998; Czerny *et al.* 1999; Yao *et al.* 2008). These genes, when deleted individually, lead to the reduction or complete loss of the compound eyes. However, when combinations of these factors (i.e. *eyg/ey*, *eyg/toe*, *toy/tsh*, and *ey/toy*) are depleted early in development the eye-antennal disc and adult head are completely lost (Hunt 1970; Yao *et al.* 2008; Zhu *et al.* 2017; Palliyil *et al.* 2018). These findings suggest that these Pax proteins play important roles in promoting growth of the entire eye-antennal disc and in specifying the fate of the developing eye.

Development of the eye-antennal disc

The suggestion that imaginal discs give rise to adult structures did not originate with studies of *Drosophila* but instead came from the developmental and anatomical analysis of butterflies, house flies, and louse flies (Swammerdam 1752; Weismann 1864; Van Rees 1884, 1888; Kowalevsky 1887; Pratt 1893, 1897). Tools to experimentally validate anatomical studies did not exist in the early years of *Drosophila* research, so no attempt appears to have been made to verify the fate of the eye-antennal disc. However, George Beadle and Boris Ephrussi developed a tissue transplantation assay that could directly address this question. They isolated eye-antennal imaginal discs from developing larvae and transplanted them into larval hosts where they would undergo metamorphosis along with the host (Fig. 3) (Beadle and Ephrussi 1935; Ephrussi and Beadle 1936). Adult heads, derived from the transplanted eye-antennal discs, were recovered from the abdomens of the host adults.

The authors developed this system to understand the nature of eye pigmentation. Eye-antennal discs that were mutant for various eye color genes were transplanted into wild-type host larvae. Interestingly, in some instances, the mutant eye-antennal disc would give rise to an adult head whose eye was of a wild-type color. These experiments provided insights into which members of the eye pigmentation pathway function autonomously within cells and which ones function at a distance and diffuse across the retina (Beadle and Ephrussi 1935, 1936a, 1936b, 1937; Howland *et al.* 1937; Ephrussi and Beadle 1937a, 1937b). Later researchers would make the conceptual leap that since transplanted discs could undergo metamorphosis and give rise to their predicted adult structures, this system could be used to shed light into tissue specification and determination (see below).

Still other researchers would go on to modify the original transplantation paradigm by implanting whole discs into adult rather than larval hosts. In these instances, the imaginal discs

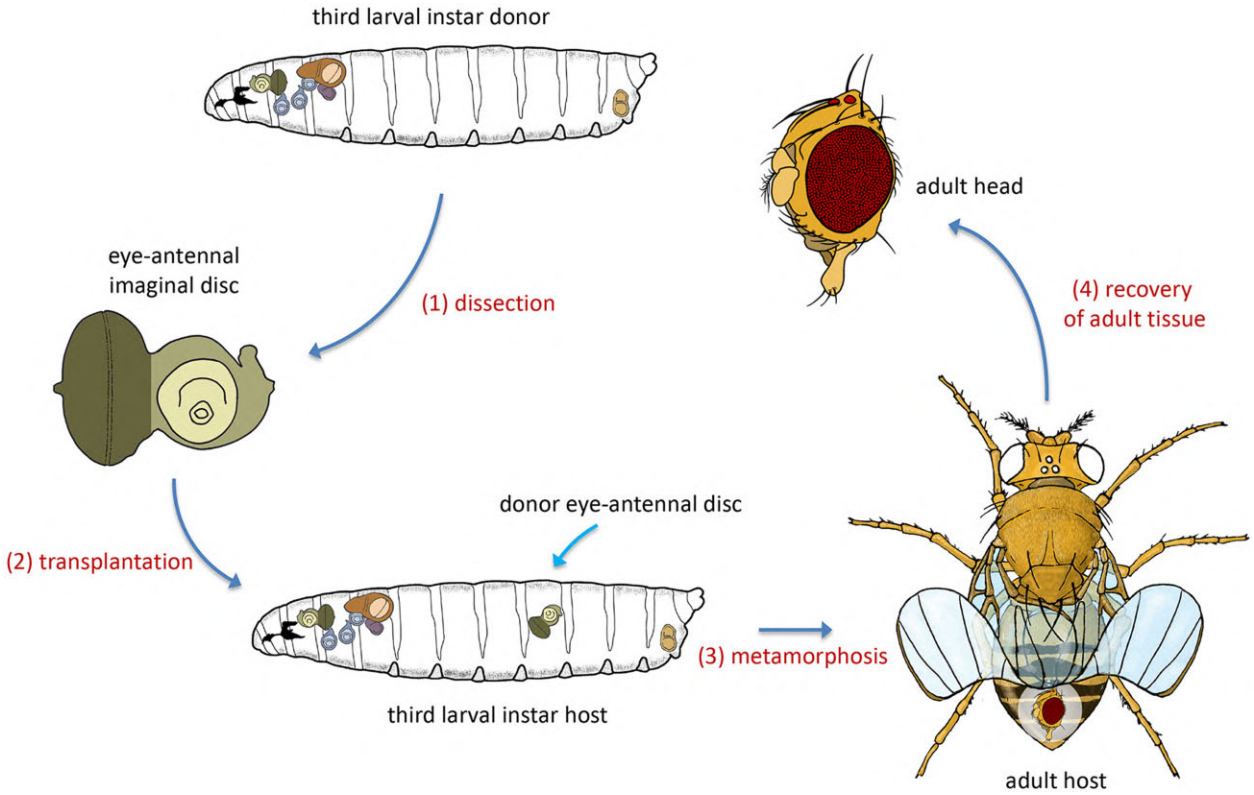

Fig. 3. Imaginal disc transplantation system. George Beadle and Boris Ephrussi developed a disc transplantation system that was later used by Ernst Hadorn to address theories of tissue determination. In this example, an eye-antennal imaginal disc is dissected and removed from a donor larva. It is then transplanted into a third instar larval host. As the host undergoes metamorphosis into an adult, the donor eye-antennal disc will be transformed into half of an adult head, which can be recovered from the abdomen of the adult fly. This schematic is idealized. Please see the papers cited within this review for the original photographs.

would continue to grow but not undergo metamorphosis (Bodenstein 1943; Ursprung 1959). Ernst Hadorn extended this method by using the adult host as an in vivo culturing system (Hadorn 1963). Discs that were serially transplanted from one adult host to another appeared to proliferate indefinitely. In fact, many of these in vivo cultures survived for several years, with some undergoing more than 300 transfers over the course of a decade. It should be noted that during these extended cultivation periods, the proliferating discs that were recovered from one adult host would have to be cut into smaller pieces before being transferred into a new adult host. This is because the glass needles that were used for the transplants had to have a small diameter so as to not create a lethal size puncture wound. The discs fragments could be removed from the adults at any time during the cultivation period and transplanted back into larval hosts where they surprisingly retained the ability to undergo metamorphosis (Hadorn 1966, 1967). Even more astounding was the fact that they "remembered" their fate and would metamorphose into their predicted adult structures.

Several researchers transplanted eye-antennal discs into larval hosts and later recovered adult head structures after both transplant and host underwent metamorphosis (Bodenstein 1938; Birmingham 1942; Vogt 1946; Schlapfer 1963; Abaturova and Ginter 1968; Ouweneel 1970c). The heads lacked the proboscis, which is, in part, how it was determined that other discs gave rise to the mouth of the fly. The origin of the proboscis was mapped to the labial and clypeo-labral discs by transplanting each of these discs into larvae and examining the metamorphosed adult derivatives (Wildermuth and Hadorn 1965; Gehring and Seippel 1967; Wildermuth 1968; Kumar *et al.* 1979). These 2 pairs of discs, along with the eye-antennal discs, are fused together during pupal development to form an intact head covering (Schoeller 1964; Milner and Haynie 1979; Milner *et al.* 1984; Haynie and Bryant 1986). Disc transplantation experiments complemented those in which the extirpation of a single eye-antennal disc from a host larva resulted in a pharate adult that lacked one half of the adult head (Birmingham 1942; Zalokar 1943). Together, these 2 types of experiments (transplantation and extirpation) provided direct and formal proof that the eye-antennal disc does indeed give rise to nearly all external adult head structures.

The transplantation of imaginal discs quickly became a powerful tool for studying development. For example, at the time it was hotly debated as to whether the information required for an imaginal disc to complete larval development was embedded with the disc itself or whether it required nonautonomous acting factors from other larval tissues such as the brain. Three studies suggested that all factors required for pushing discs through larval development are contained within the eye-antennal disc itself and are not secreted from other larval tissues. One study showed that a young eye-antennal disc could complete larval development if transplanted into adult hosts (Garcia-Bellido 1965). And 2 other reports demonstrated that even young embryos, when dissociated, aggregated, and transplanted into adults, could complete both embryogenesis and larval development (Hadorn 1968; Schubiger *et al.* 1969). It seemed that extrinsic factors from other larval tissues were not needed for an imaginal disc to develop. These studies, although long forgotten, are still relevant today.

Several recent papers have suggested that communication between the brain and imaginal discs are essential during larval development to ensure that each disc completes development, reaches its final size prior to pupariation, and maintains right-left growth symmetry (Colombani *et al.* 2012; Garelli *et al.* 2012; Colombani *et al.* 2015; Vallejo *et al.* 2015; Boulan *et al.* 2019). However, the disc transplantation studies described above suggest that imaginal disc development might be more akin to building an airplane. Each portion of the plane, be it a wing or an engine, is built by machinists (using blueprints) without the need of having to constantly consult with upper management. From this perspective, the instructions for building each adult structure such as the eye, antenna, wing, leg, haltere, and genitalia may be embedded solely within the imaginal disc itself so that inter-organ communication during larval stages is not necessarily required for discs to complete development. Two additional experiments support this interpretation, First, a young immature imaginal disc when transplanted into an adult abdomen would apparently stop growing when it reached the size of a normal disc that had completed larval development (Bryant and Levinson 1985). Second, cells taken from the blastoderm stage embryos and implanted into adult abdomens would develop into imaginal discs (Gehring 1970; Chan and Gehring 1971). These 2 experiments suggested that nonautonomous signals form larval tissues are not required for determination, specification, and growth control.

Determination of imaginal disc fate

Researchers were quick to realize the potential that disc transplantation had in solving a wide range of problems in developmental biology. Two of the most vexing problems concerned the timing of when and mechanisms by which a region of the embryo eventually committed itself to forming an adult structure—this is the process of determination. Studies in vertebrates had suggested that tissue fate was determined during embryogenesis. Cells from different parts of the embryo were dissociated, isolated, mixed, and transplanted back into host embryos. The recovered tissues were able to reconstruct their original fates and produce the predicted organs with no changes in fate. This suggested that tissue fate was "determined" at the time of the dissociation, which in those experiments was during embryogenesis (Holtfreter 1943; Moscona and Moscona 1952; Moscona 1957; Weiss and Taylor 1960; Trinkaus and Gross 1961).

Taking a cue from these studies, researchers first took cells from different imaginal discs and similarly dissociated, mixed, transplanted them into host larvae, and recovered both host and the transplant after both had undergone metamorphosis. In the first experiments of this kind, cells from 2 genetically distinct third larval instar wing imaginal discs were mixed, transplanted into adult hosts to first promote growth, and then transplanted into larval hosts to force metamorphosis. The resulting adult wing structure was a mosaic of both genotypes suggesting that the imaginal discs were determined at the time of the dissociation (Hadorn *et al.* 1959; Ursprung and Hadorn 1962). Later attempts would mix dissociated cells from distinct types of imaginal disc cells (i.e. wing and antenna). A wide range of combinations that included wing/antenna, leg/antenna, wing/haltere, genital/wing, and wing/leg imaginal discs were tested. In each experiment, the cell mixtures did not give rise to mosaic adult tissues as had happened when the 2 cell populations came from the same type of disc. Instead, the 2 cell populations separated from each other and formed structures that were autotypic of their original fate. The ability of dissociated cells to retain their distinct cell affinities and adopt their original fate showed again the fate of each imaginal disc had been determined by the third larval instar (Nothiger 1964; Garcia-Bellido 1966; Gehring 1966; Tobler 1966; Garcia-Bellido 1968; Tobler and Schaerer 1971).

Once a method for dissociating, mixing, and culturing embryonic cells was developed, research began in earnest to see if the fate of imaginal discs were, like vertebrate tissues, determined during embryogenesis. Embryos were bisected along the anterior-posterior axis, cultured in adults, and then forced to undergo metamorphosis after being transplanted into larval hosts. Each half of the embryo produced imaginal discs and adult structures that would be expected from that region of the embryo (Hadorn *et al.* 1968; Hadorn and Muller 1974). Gerold Schubiger then isolated cells from anterior half of stage 10 fly embryos and mixed them with cells from the posterior half of genetically distinct embryos of the same stage. Imaginal discs could be recovered if the cell mixtures were cultured in adult hosts. These discs would then differentiate if transferred to larval hosts and allowed to undergo metamorphosis. Since the anterior and posterior embryonic cells carried different genetic markers, it was possible to correlate the imaginal discs and adult structures with their embryonic origin. Head structures such as the eye and antenna could only be produced by anterior embryonic cells (Schubiger *et al.* 1969). These results suggested that the fate of the eye-antennal disc is determined by embryonic stage 10.

Walter Gehring pushed the conceptual boundaries of fate determination and specification even further by showing that head structures would develop if anterior cells from blastoderm embryos (stage 3) were isolated and cultured as described above (Fig. 4) (Gehring 1970; Chan and Gehring 1971). At this point in development, *toy* is expressed in a band of cells within the anterior-dorsal quadrant of the embryonic head (Czerny *et al.* 1999; Blanco and Gehring 2008). This region of the embryo will ultimately give rise to the optic lobes, the Bolwig organ, and the eye portion of the eye-antennal disc (Green *et al.* 1993; Younossi-Hartenstein *et al.* 1993). The onset and spatial localization of *toy* expression in this region results from interplay between the Bicoid (Bcd), Torso (Tor), and Dorsal (Dl) maternally contributed signaling systems and zygotically activated factors including Hunchback (Hb), Knirps (Kni), and Decapentaplegic (Dpp) (Blanco and Gehring 2008). Together, these findings suggest that the fate of the eye-antennal disc is sealed by the time the embryo has reached the blastoderm stage of development (Gehring 1970; Chan and Gehring 1971; Czerny *et al.* 1999; Blanco and Gehring 2008).

Fate mapping of the eye-antennal disc

Peter Bryant's landmark treatise on postembryonic development of *Drosophila* described more than fifty major physical landmarks on the adult head (Bryant 1978). While the transplantation of whole eye-antennal discs made it clear that the eye-antennal disc gives rise to all head structures minus the proboscis, a detailed fate map of the eye-antennal disc itself was missing. In other words, it was not clear, with some major exceptions such as the eye and antenna, which portion of the eye-antennal disc gave rise to each of the 50 plus adult structures. A breakthrough came when it was discovered that fragments of imaginal discs could be transplanted into larvae and recovered from adults (Fig. 5). As with whole discs, fragments of imaginal discs would also undergo metamorphosis along with the larval host (Hadorn *et al.* 1949; Ursprung 1959; Hadorn and Buck 1962; Schubiger

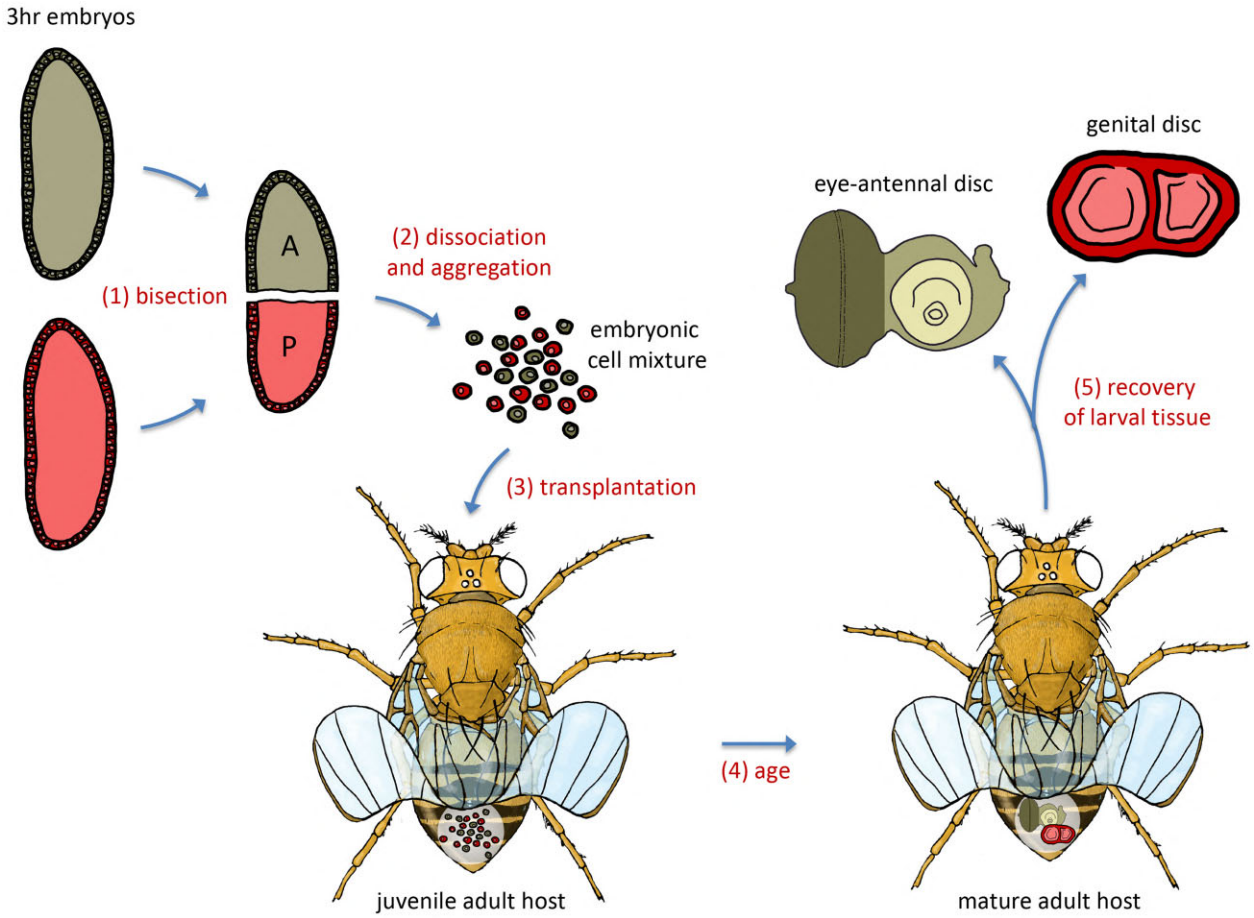

Fig. 4. Imaginal disc fate in Drosophila is determined during embryogenesis. Embryos of different genotypes are first bisected. The anterior of one type of embryo was mixed with the posterior half of a different embryo type. The cells are then dissociated and then mixed to form an aggregate which are then transplanted into host adults. As the adults age, the transplanted cells would give rise to imaginal discs. A recovered eye-antennal disc was comprised of cells from the anterior half of the embryo. A, anterior; P, posterior. This schematic is idealized. Please see the papers cited within this review for the original photographs.

1971). The transplantation of disc fragments was an important experimental advance because the fragments would metamorphose into adult structures that were appropriate for their position within the eye-antennal disc (Fig. 6). Furthermore, a fragment could regenerate the missing pieces of the disc that had been discarded. For example, if a fragment containing just the antennal portion of the disc was transplanted into larva, then the resulting adult head would contain the antenna, maxillary palpus, and portions of head epidermis but it would lack the compound eyes and ocelli (Gehring 1966). Conversely, if just the eye portion of the disc were transplanted, then the resulting adult head would contain a compound eye, an ocellus, and a different part of the head epidermis but both antenna and maxillary palpus would be missing (Lebovitz and Ready 1986). Cutting of the eye-antennal disc at different positions and transplanting the resulting fragments allowed for a relatively detailed map of the eye-antennal disc to be generated (Gehring 1966; Ouweneel 1970c; Haynie and Bryant 1986; Lebovitz and Ready 1986). Similar studies using fragments of other imaginal discs produced fate maps for the wing, leg, genital, haltere and labial discs as well (Hadorn and Gloor 1946; Hadorn *et al.* 1949; Ursprung 1957; Loosli 1959; Ursprung 1959; Hadorn and Buck 1962; Schubiger 1968; Ouweneel 1970a; Schubiger 1971).

Regional organization of the eye-antennal disc

The fine fate map of the eye-antennal disc established that it is divided into specific territories from which individual adult structures are derived. These findings were consistent with the phenotypic analysis of loss-of-function mutants such *eyeless* and *ocelliless* in which specific structures on the adult head are missing. Together, these findings prognosticated the discovery of gene regulatory networks that are expressed in spatially restricted domains. The most celebrated example is the retinal determination network, which controls eye specification. The most anterior region of the eye field is where all known members of this network, including *eyeless*, are expressed. These factors are responsible for specifying the fate of the compound eye (Kumar 2010). Their loss leads to the severe reduction or loss of the eye (Fig. 7a–c) (Hoge 1915; Milani 1941; Ives 1942; Hunt 1970; Sved 1986; Bonini *et al.* 1993; Cheyette *et al.* 1994; Mardon *et al.* 1994; Kronhamn *et al.* 2002). Conversely, forced expression of these factors in nonretinal tissues such as the antenna, wing, halters, and leg imaginal disc is sufficient to induce their transformation into ectopic compound eyes (Fig. 7d) (Halder *et al.* 1995; Bonini *et al.* 1997; Pignoni *et al.* 1997; Shen and Mardon 1997; Pan and Rubin

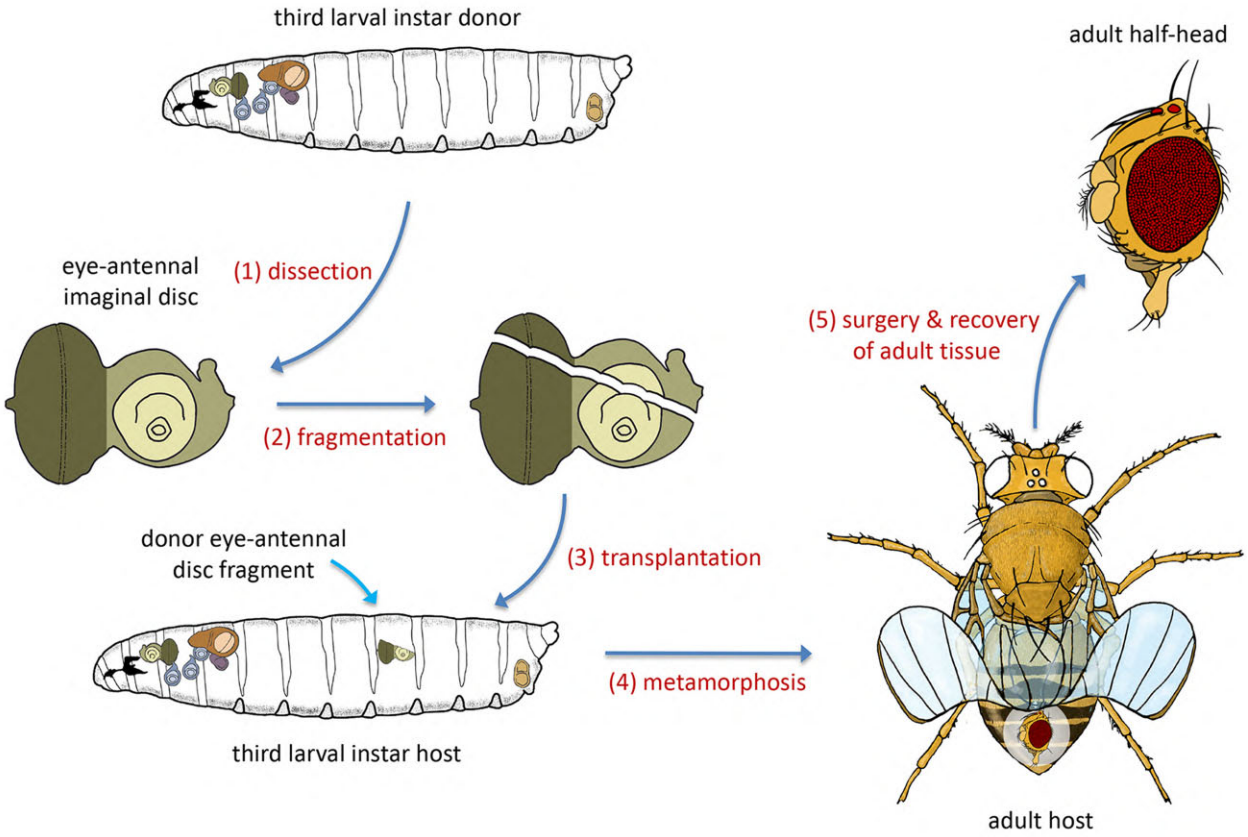

Fig. 5. Determination of *Drosophila* imaginal discs. Ernst Hadorn used the larval disc transplantation system developed by George Beadle and Boris Ephrussi to test theories of tissue determination. In this example, an eye-antennal disc is removed from a donor larva, fragmented, and transplanted into a larval host. Upon metamorphosis the fragment will regenerate the lost parts of the disc will give rise to half of the adult head. Since the identity of the eye-antennal disc and the adult head is synchronized, the identity of the tissue is said to have been *determined* at the time of transplantation. This schematic is idealized. Please see the papers cited within this review for the original photographs.

1998; Czerny *et al.* 1999; Singh *et al.* 2002; Jang *et al.* 2003; Weasner *et al.* 2007; Braid and Verheyen 2008; Yao *et al.* 2008; Bessa *et al.* 2009; Datta *et al.* 2009). Both phenotypes indicated that these factors function as selector genes for eye formation. This term was coined by Antonio Garcia-Bellido to describe early acting genes that specified the fate of the wing (Garcia-Bellido 1975). Within the retinal determination network lies a core module consisting of the *twin of eyeless (toy)*, *eyeless (ey)*, *sine oculis (so)*, *eyes absent (eya)*, and *dachshund (dac)* genes (Fig. 7e). These are the fly orthologs of vertebrate Pax6, Six1/2, Eya1-4, and Dach1/2. This core module controls eye development in both invertebrates and vertebrates. It also controls the specification of several tissues outside the eye in *Drosophila*, mice, zebrafish, and humans.

The existence of the retinal determination network and their spatial expression patterns were, unbeknownst to the authors of the above studies, predicted by disc fragmentation and transplantation experiments. Transplanted fragments containing just the antenna and the most anterior part of the eye field would astonishingly metamorphose into adult heads that contained the compound eye. This occurred even though the transplanted disc fragment lacked developing photoreceptor clusters or the morphogenetic furrow (Fig. 5) (Lebovitz and Ready 1986). Both cellular elements are known today to be essential for continued patterning of the eye field. Although unknown at the time, the retinal determination network is functioning in the anterior portion of the eye and guiding cells toward adopting a retinal fate. As such, the disc transplants did in fact have the potential to metamorphose into eyes. Later in this chapter we will discuss potential mechanisms by which pattern formation could re-initiate in these fragments despite the absence of the morphogenetic furrow and photoreceptor clusters.

Maintaining the fate of the eye-antennal disc

Several transformations listed above are examples of intra-disc changes in fate. The young eye-antennal disc appears to be a chaotic environment with multiple gene regulatory networks competing for primacy. There are networks for each of the main structures within the disc and it appears that each one plays both offensive and defensive roles. In other words, the same factors that are required to promote one fate appear to also repress the adoption of other fates. For example, 2 members of the retinal determination network, *sine oculis (so)* and *eyes absent (eya)* are required simultaneously for the promotion of eye identity and the repression of head fate. While the compound eye is eliminated in loss-of-function mutants their loss also leads to the derepression of genes that control head epidermis fate and the eye is transformed into epidermal tissue (Milani 1941; Sved 1986; Bonini *et al.* 1993; Weasner and Kumar 2013). Likewise, while either gene can induce the formation of ectopic eyes when expressed in nonocular tissues, they do so in part by repressing the expression of endogenous selector genes (Bonini *et al.* 1997;

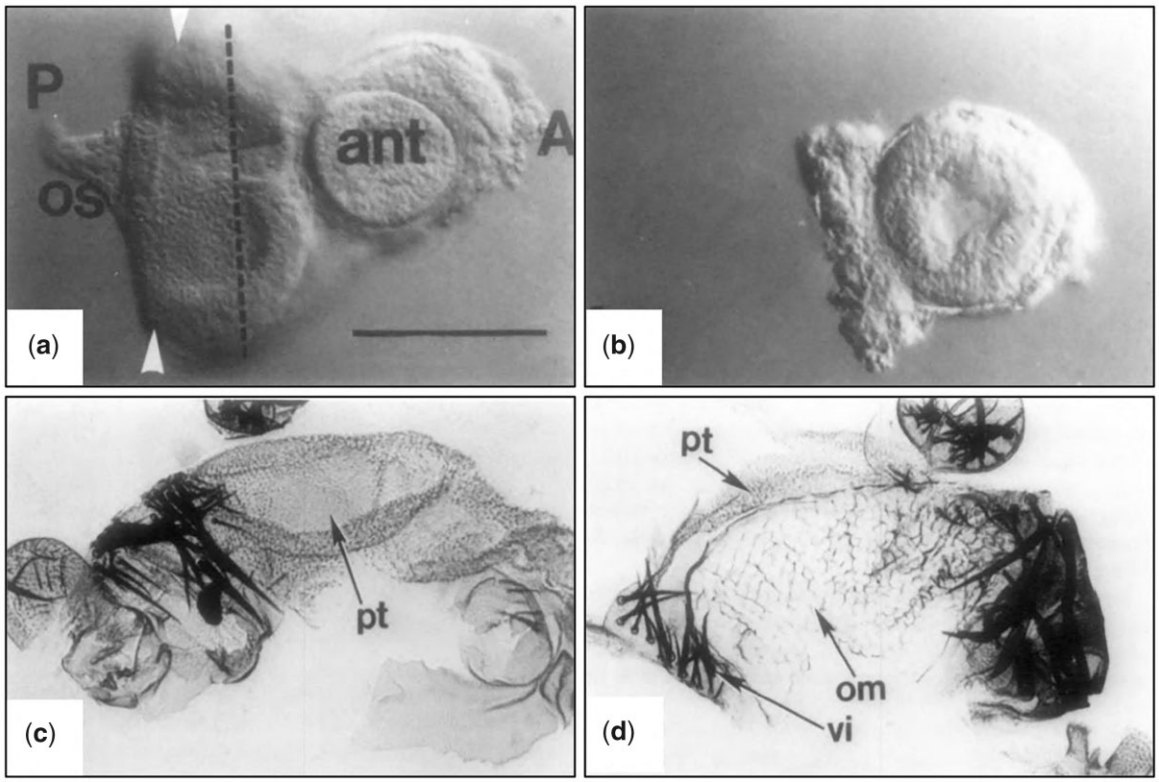

Fig. 6. Fragments of the eye-antennal disc give rise to adult head structures. a–d) Light microscope images taken from Lebovitz and Ready (1986). a) A wild-type eye-antennal disc showing position of future cut. b) A fragment of the eye-antennal disc containing just the antenna and anterior part of the eye disc. This fragment lacks photoreceptor clusters and the morphogenetic furrow. It will be transplanted into donor adults and larva as shown in Fig. 7. c, d) Adult tissue recovered from the abdomens of adults after metamorphosis.

Pignoni *et al.* 1997; Weasner *et al.* 2007; Anderson *et al.* 2012). For example, when the So-Eya complex induces ectopic eyes within the antenna, it does so, in part, by inhibiting the expression of *cut* (*ct*), *Distalless* (*Dll*), and *Lim1*.

Similarly, the Exd-Hth complex is required to prevent a reciprocal change in fate. The complex normally promotes both head epidermis and antennal fate (Casares and Mann 1998). When the complex is disrupted within the head epidermis, the retinal determination network is ectopically activated, and the cuticle is transformed into an ectopic compound eye (Gonzalez-Crespo and Morata 1995; Pai *et al.* 1998; Pichaud and Casares 2000). In contrast, over-expression of the complex within the eye field completely inhibits retinal development and induces its transformation into head epidermis (Pai *et al.* 1998; Singh *et al.* 2002). The Wingless (Wg) seems to play a similar role in preventing nonocular tissues within the eye-antennal disc from adopting an eye fate. Disrupting this pathway within the dorsal head epidermis that borders the eye field results in the transformation of head epidermis into retinal tissue (Ma and Moses 1995; Treisman and Rubin 1995; Royet and Finkelstein 1996, 1997; Maurel-Zaffran and Treisman 2000; Oros *et al.* 2010). Conversely, hyperactivation of Wg signaling within the eye field induces a homeotic transformation into head epidermal tissue (Ma and Moses 1995; Treisman and Rubin 1995). Other intra-disc transformations such as eye-to-antenna are caused by a hyperactivation of EGF Receptor signaling or a loss of Notch activation (Kumar and Moses 2001a). Likewise, the head epidermis-to-antenna, and maxillary palpus-to-antenna are attributed to the loss of the NURF nucleosome remodeling complex and a hyperactivation of Wingless (Wg) signaling, respectively (Lebreton *et al.* 2008; Ordway *et al.* 2021).

Transdetermination

One of the truly unexpected and astonishing observations was that portions of genital disc fragments would on occasion differentiate into other imaginal discs. The adult tissues were mosaics of adult genitals and most often either antennae or legs. These genital-to-leg and genital-to-antenna transformations took place even if the transplanted fragments had adopted a genital fate in previous transfers. These allotypic changes in the determined state of the disc were coined *transdetermination* events (Hadorn 1963). Such occurrences were not limited to the genital disc. In fact, portions of labial, leg, haltere, wing, and eye-antennal disc fragments were also recorded as undergoing changes to their original determined state (Fig. 8) (Schlapfer 1963; Gehring 1966; Tobler 1966; Perriard 1967; Gehring *et al.* 1968; Schubiger 1968; Schubiger and Hadorn 1968; Wildermuth 1968). Over many years, a detailed map of transdetermination events emerged—it documented which types of fate changes were possible (Hadorn 1968, 1978). Each disc had a propensity to change its fate that was both quantitatively and qualitatively different from other discs. For example, while some discs can transdetermine into many different disc types, other discs have a more limited capacity to have their fate redirected. An example of the former is the antennal disc, which can transdetermine and give rise to adult genital, labial, leg, and wing structures. In contrast, the eye disc can only have its fate redirected toward that of a wing. Similarly, the frequency of each type of fate change varies considerably. The most common transdetermination events to be recovered are the labial-to-antenna, genital-to-antenna, and leg-to-wing disc switches. On the other end of the spectrum, antenna-to-leg, wing-to-leg, and eye-to-wing transformations are rare events.

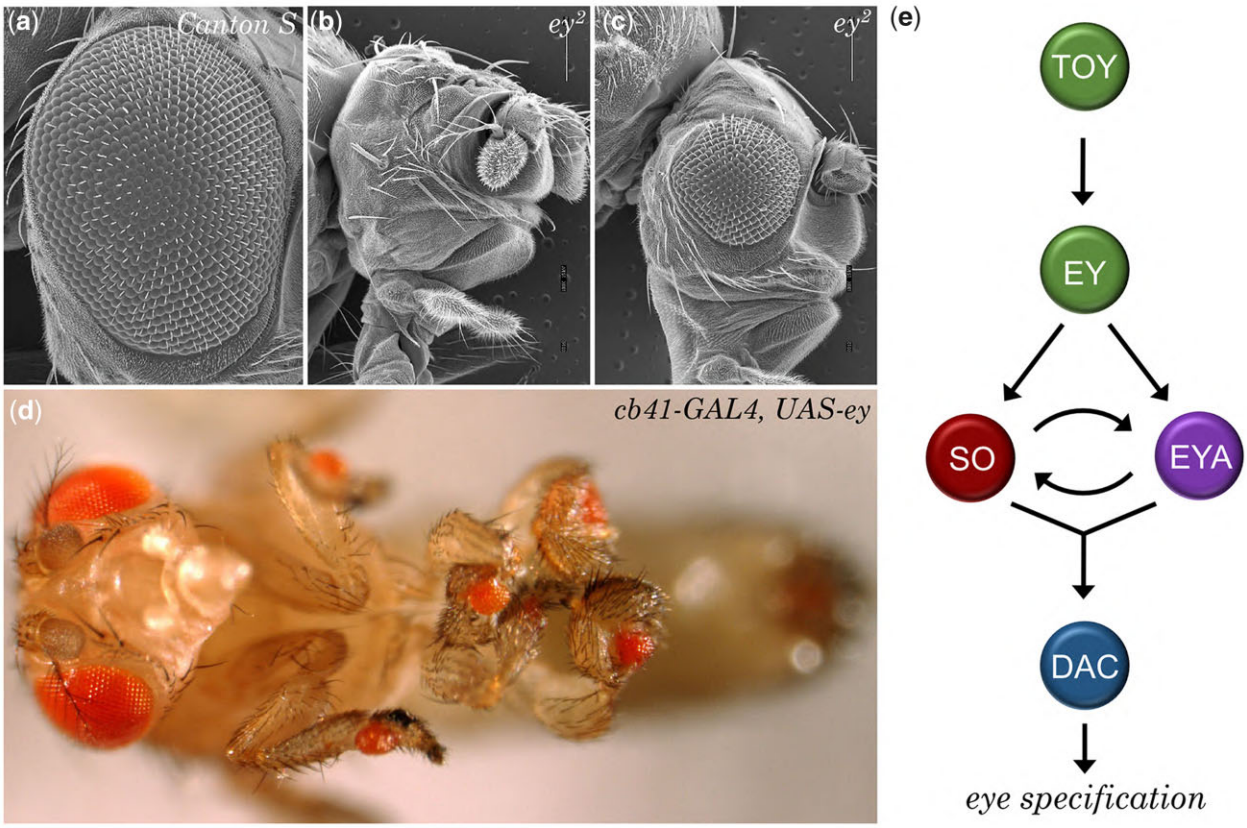

Fig. 7. The retinal determination network of Drosophila. a) Scanning electron micrograph (SEM) of a wild-type compound eye. b, c) SEM images of loss-of-function *eyeless* mutants in which the eyes are either completely missing (b) or severely reduced in size (c). d) Forced expression of *eyeless* in nonretinal tissues results in the formation of ectopic eyes. e) The core members of the retinal determination network are depicted and include a set of DNA binding proteins and transcriptional activators. Depicted are 2 Pax6 transcription factors Twin of Eyeless (Toy) and Eyeless (Ey), the SIX protein homolog Sine Oculis, the Eyes Absent (Eya) transcriptional activator/phosphatase, and Dachshund (Dac) a member of the Ski/Sno family of transcriptional repressors.

And lastly, while some transdetermination events are reversible (antenna-to-leg-to-antenna and eye-to-wing-to-eye), others only occur unidirectionally (haltere-to-wing). Overall, these experiments provided invaluable information on the multipotency of imaginal discs (Fig. 9).

A genetic and molecular understanding of how the determined state of the disc is established came from mutants that display similar, if not identical, changes in tissue fate. The greatest contribution has come from mutants that mimic the antenna-to-leg and eye-to-wing transdetermination events. Insight into the former has come from studies of the Hox gene, *Antennapedia (Antp)*. In loss-of-function mutants, the second thoracic leg is transformed into an antenna while the reciprocal transformation (antenna-to-leg) occurs within a series of dominant gain-of-function mutations (Le Calvez 1948a, 1948b, 1948c; Yu 1949; Denell 1973; Denell *et al.* 1981; Struhl 1981b). During normal development, *Antp* expression is absent from the eye-antennal disc but it is ectopically activated in gain-of-function mutants (Wirz *et al.* 1986; Jorgensen and Garber 1987). Its role in promoting leg development was confirmed when forced expression of *Antp* within the antenna transformed it into a leg (Schneuwly *et al.* 1987a, 1987b). Thus, the decision to become an antenna or leg depends on whether *Antp* expression is activated or repressed.

The repression of *Antp* within the eye-antennal disc (as well as within the embryo) is mediated by *Polycomb (Pc)*, the founding member of the Polycomb group (PcG) of epigenetic repressors. At the molecular level, it binds, reads, and interprets the methylation of lysine 27 on histone H3 (H3K27me3) which is laid down earlier by the Enhancer of zeste (Ez) protein (Lewis 1947; Czermin *et al.* 2002; Müller *et al.* 2002; Min *et al.* 2003). The same antenna-to-leg transformation that is seen in *Antp* gain-of-function mutants also occurs in viable dominant *Pc* mutants (Lindsley and Grell 1968; Denell 1978). Additionally, combining *Pc* loss and *Antp* gain-of-function mutant alleles together increases the severity and frequency of antenna-to-leg homeotic transformations (Bulyzhenkov *et al.* 1975). At the molecular level, Pc directly interacts with histones at the *Antp* promoter and represses its expression (Zink *et al.* 1991; Bantignies *et al.* 2011). In the absence of Pc-mediated repression, *Antp* expression is ectopically activated within the antennal field, which, in turn, triggers the transformation of the antenna into a leg (Zhu *et al.* 2018).

The transformation of the antenna into legs is not limited to *Antp* and *Pc* alleles but is also observed in *homothorax (hth)* and *extradenticle (exd)* loss-of-function mutants (Casares and Mann 1998; Pai *et al.* 1998). These proteins form a complex (Exd-Hth) that helps confer target selectivity to Hox proteins that, on their own, have very low DNA-binding specificities. Within the T2 segment the Exd-Hth complex allows for Antp to regulate target genes that are essential for T2 leg and wing specification. Likewise, in the T3 segment Exd-Hth will interact with another Hox protein Ultrabithorax (Ubx) to specific the fate of the T3 leg and haltere (Chan *et al.* 1994; Rieckhof *et al.* 1997; Ryoo *et al.* 1999; Lelli *et al.* 2011; Slattery *et al.* 2011a, 2011b). Similar Hox-Exd–Hth interactions take place within all segmental units of the fly.

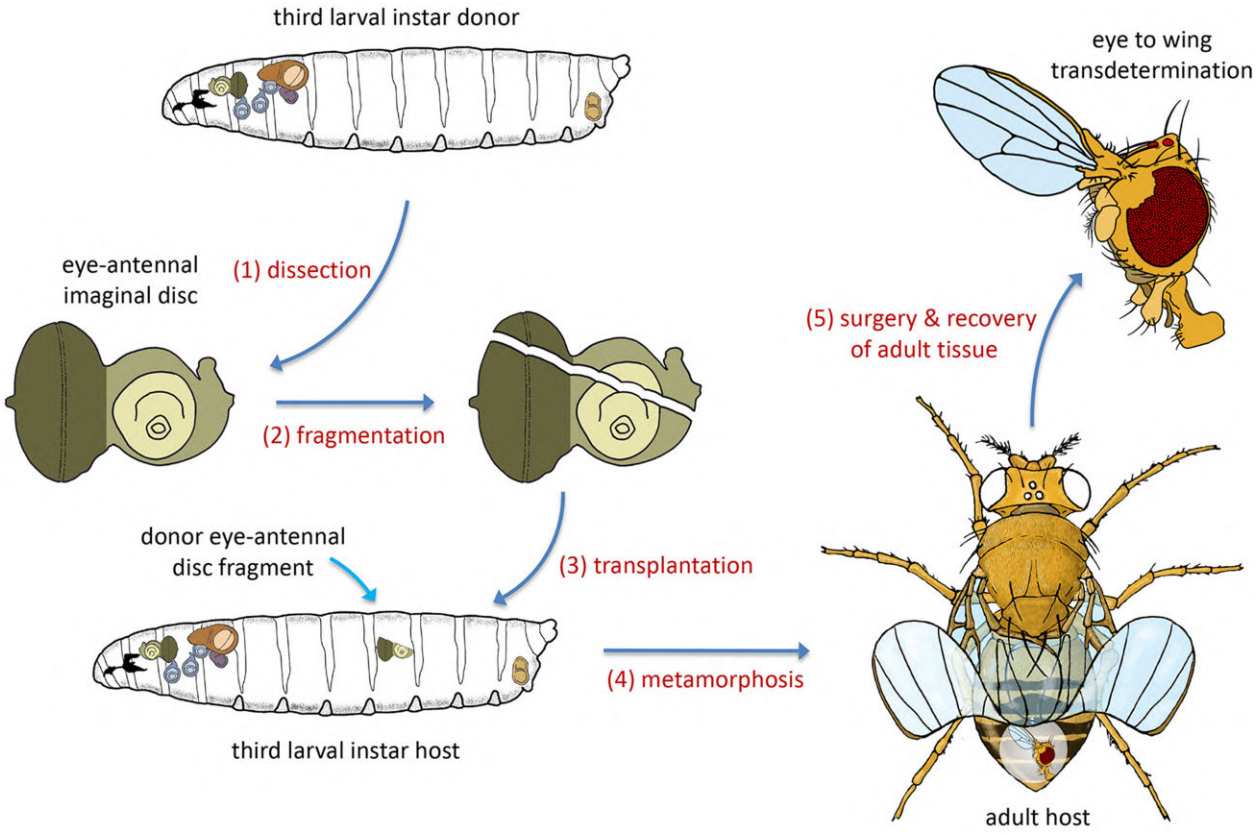

Fig. 8. Transdetermination of *Drosophila* imaginal discs fragments. In this paradigm, Ernst Hadorn fragmented imaginal discs and transplanted small pieces of the discs into host larvae. Under most circumstances, these fragments would regenerate and give rise to the appropriate adult structure (see Fig. 6 for an original image from Lebovitz and Ready, 1986). However, in a small number of instances, the imaginal disc would produce adult structures that would normally be derived from different imaginal discs. In this example, the regenerating portion of an eye-antennal disc fragment would *transdetermine* into a wing. The resulting adult tissue is a mosaic of head and wing tissue. Loss of several gene mimic this transdetermination event. This schematic is idealized. Please see the papers cited within this review for the original photographs.

Studies of *Antp* have also been informative for understanding how the eye adopts its primary fate. It appears that inhibiting its expression is important for preventing the eye from being transformed into a wing. A dominant gain-of-function allele ($Antp^{Ctx}$) mimics the transdetermination of the eye to wing seen in disc transplantation assays (Scott *et al.* 1983; Prince *et al.* 2008). Interestingly, not all dominant *Antp* gain-of-function alleles show this specific fate transformation nor does the simple over-expression of *Antp* (Kurata *et al.* 2000). It suggests that another molecular lesion might be reside within the $Antp^{Ctx}$ stock. Interestingly, if *Antp* gain-of-function alleles are combined with a null mutant of *toy*, then the eye does in fact transdetermine into a wing (Gehring *et al.* 2009). This fate switch can also be induced if *Antp* is over-expressed within eye discs that are also subjected to hyperactivation of the Notch signaling pathway (Kurata *et al.* 2000; Papadopoulos *et al.* 2011). Both results imply that for Antp to induce an eye to wing transformation, it must be accompanied by a secondary mutation that increases multipotency of the disc.

As with the antenna, PcG group proteins appear to repress *Antp* expression within the eye disc as well. When Pc levels are depleted from the eye disc, *Antp* expression is ectopically activated and the eye is transformed into a wing (Zhu *et al.* 2018). However, as described above, the presence of *Antp*, on its own, appears insufficient to induce the eye-to-wing fate switch. This is based, in part, on the observation that the reduction or loss of the remaining PcG proteins also leads to the activation of *Antp* but has little effect on the fate of the eye. But, if Ey or Toy levels are reduced along with PcG members, then the eye is transformed into a wing (Zhu *et al.* 2018). Since PcG proteins regulate *Antp* expression, the combined loss of PcG and Pax6 might be genetically and mechanistically similar to the over-expression of Antp and loss of Pax6.

A key determinant of wing identity is the selector gene *vestigial* (*vg*). Its role in wing development rivals that of Toy and Ey within the developing eye. *vg* loss-of-function mutants lack most wing blade structures while forced expression of *vg* within the developing eye is sufficient to induce a transformation of the eye into a wing (Waddington 1940; Williams *et al.* 1991; Kim *et al.* 1996; Simmonds *et al.* 1998). *vg* appears to occupy a special position within the path that imaginal disc precursors take on the way to choosing between an eye and wing fate. While *vg* is normally expressed within the developing wing disc its expression is repressed in the developing eye-antennal disc by PcG-mediated epigenetic silencing (Ahmad and Spens 2019). When Pc levels are knocked down in the eye-antennal disc, the inhibition of *vg* expression is relieved just within the eye field (Zhu *et al.* 2018). The same is true of eye discs in which both *Antp* expression and Notch signaling are simultaneously hyperactivated (Kurata *et al.* 2000). As the forced expression of *vg* transforms the eye into a wing, the ectopic activation of *vg* expression is the likely cause of the eye to wing transformation when Pc, Antp, and Notch levels/activity are manipulated. Based on these observations, eye disc fragments that transdetermine into wings are predicted to also do so via the gain of *vg* repression within the eye field. One could

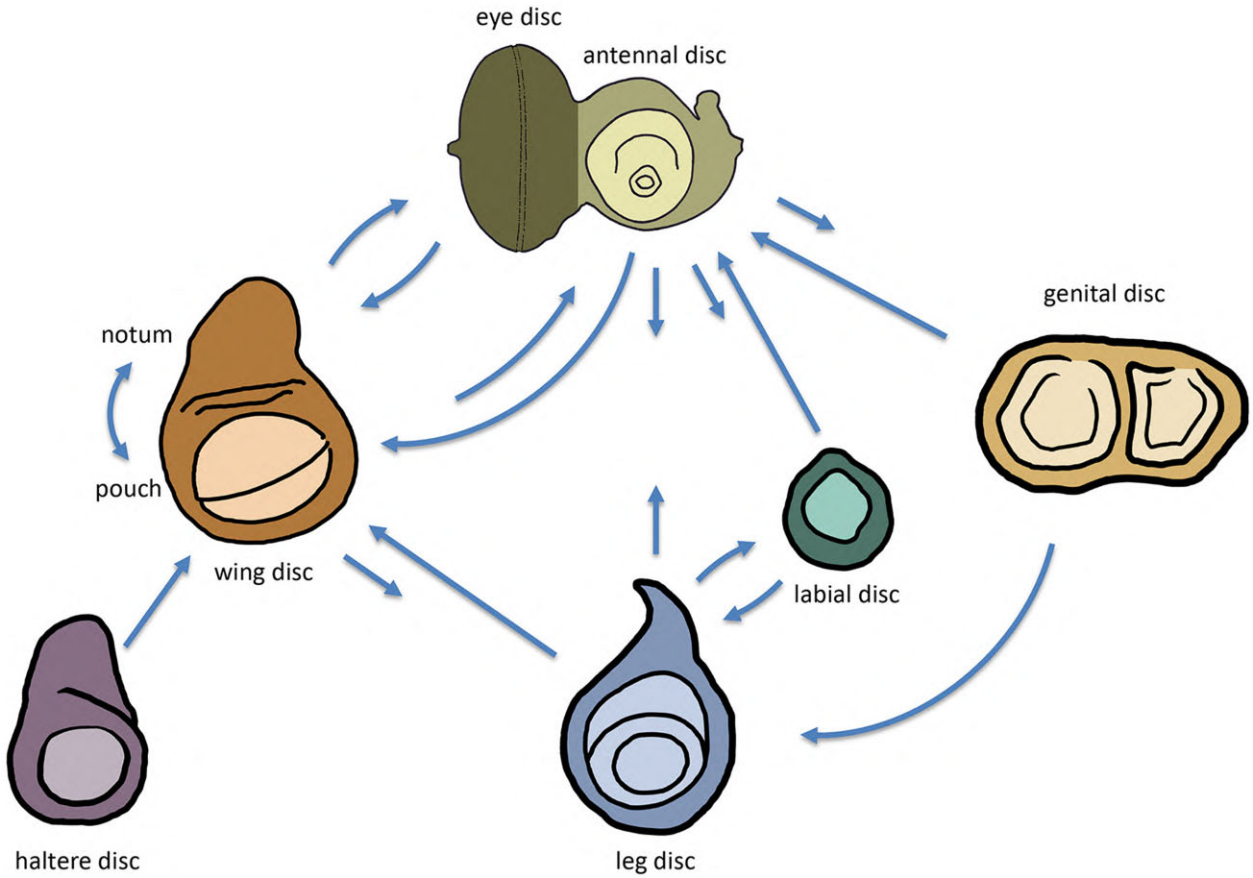

Fig. 9. Map of transdetermination events. The ability of each imaginal disc to transdetermine into another disc is quantitatively and qualitatively unique when compared to all other imaginal discs. For example, the antennal portion can transdetermine into wing, leg, labial, and genital discs while the eye portion can *only* adopt the fate of the wing disc. Some transdetermination events are unidirectional (i.e. haltere-to-wing) while others are bidirectional (antenna-to-leg and leg-to-antenna). And some events occur at relatively high frequencies (i.e. leg-to-wing) while others are rare events (i.e. antenna-to-genital).

envisage that a similar activation of *vg* expression in the eye field might also be the underlying cause for when the eye to transforms into a wing when *winged-eye (wge)* is over-expressed or when transcription of the *loboid (ld)*, *ophthalmoptera (opht)*, *eyes-reduced (eyr)*, and *Deformed (Dfd)* genes is compromised in the eye-antennal disc (Goldschmidt and Lederman-Klein 1958; Edwards and Gardner 1966; Kobel 1968; Ouweneel 1969a, 1969b, 1970a, 1970b; Postlethwait 1974; Merrill *et al.* 1987; Katsuyama *et al.* 2005; Masuko *et al.* 2018).

For reasons that are not entirely clear only the dorsal-anterior quadrant of the eye field transdetermines into a wing in nearly all situations described in the last few paragraphs. The only exception is the case of forcibly expression *vg*, which results in multiple wings begin generated throughout the eye field. Why the discrepancy in ectopic wing position? It has been noted that in cases where Pc levels are reduced or both Antp and Notch levels/activity are hyperactivated, *vg* is only found within the dorsal-anterior quadrant of the eye (Kurata *et al.* 2000; Zhu *et al.* 2018). In contrast, enhancers that drive expression throughout the entire eye disc at some point in development are usually used to forcibly express *vg*. It remains an open question why manipulations of Pc, Antp, and Notch, which also take place throughout the disc, do not activate *vg* expression more broadly.

Although wild-type eye discs, when fragmented and transplanted into hosts, were only observed to transdetermine into wings, a wide range of additional homeotic transformations are recovered in loss-of-function mutants. For example, in addition to adopting a wing fate, the eye has been recorded as being transformed into an abdomen (Postlethwait *et al.* 1972), a leg (Waddington and Pilkington 1943; Kurata *et al.* 2000), a maxillary palp (Waddington 1942; Postlethwait *et al.* 1972), an antenna (Waddington 1942; Kumar and Moses 2001a; Duong *et al.* 2008), and into portions of the head epidermis (Weasner and Kumar 2013).

The duration of culture within adult hosts can also be critical for uncovering tissue fate choices. For example, if wild-type eye discs are cultured in adult hosts for 12 days prior to transplantation into larvae, then the eye can transdetermine only into the wing notum. But an additional 3 days in adult culture results in the transformation of the eye into the wing blade (Schmid 1985). Since the wing pouch can transdetermine into the notum and vice versa (Hadorn 1968, 1978), it makes sense that the fragments of the eye would adopt the fate of either part of the wing. These results also suggest that the choice that a nascent imaginal disc must make in terms of adopting either an eye-antennal or wing imaginal disc fate are more developmentally nuanced than is current predicted. As such, new studies in which eye-antennal disc fragments are subjected to a wide-range of culturing conditions might shed new light on the steps that a burgeoning imaginal disc takes on its path toward its final fate. This should be a

viable avenue given the significant advances that have been made in tissue culture systems.

Interestingly, the eye-to-leg homeotic transformation recorded by Waddington and Pilkington is also chronicled as a transdetermination event but it only occurs if the transplanted eye disc fragment comes from an *ophthalmoptera* (*opht*) mutant instead of a wild-type donor larva (Schmid 1985). The implication of this intriguing result is that transplantation of eye-antennal disc fragments might still prove valuable in unlocking the secrets of tissue specification if it is combined with modern molecular tools that can disrupt gene expression. For instance, transplanting discs in which individual transcription factors or signal pathway components have been depleted by RNAi could reveal developmental choices that have escaped detection.

How does a fragment of one imaginal disc transdetermine and give rise to adult structures that are normally derived from a completely different imaginal disc? The simple answer is that something goes wrong during an attempt by the fragment to regenerate itself and replace the lost tissue. Soon after an imaginal disc is fragmented, localized cell proliferation (called regenerative proliferation) takes place along the edge of the fragment that has been wounded by the fragmentation (French *et al.* 1976; Abbott *et al.* 1981; Bryant *et al.* 1981; Dale and Bownes 1981; O'Brochta and Bryant 1987). The zone of regenerative proliferation is called a blastema. When the blastema is transplanted by itself into an adult abdomen it can fully regenerate the tissue that was removed from the transplanted fragment (Karpen and Schubiger 1981). Modern lineage tracing methods have confirmed that the regenerated tissue comes from the blastema (Bosch *et al.* 2008; Smith-Bolton *et al.* 2009; Sustar *et al.* 2011; Herrera *et al.* 2013; Worley *et al.* 2013). These studies indicate that all molecular information for proper fate specification and patterning is present within the blastema itself.

In recent years, several very clever efforts to meld clonal analysis with cell ablation technologies have given modern day researchers an array of tools to identify the molecular mechanisms underlying tissue regeneration (Sustar and Schubiger 2005; Smith-Bolton *et al.* 2009; Bergantinos *et al.* 2010; Cohen *et al.* 2018; Harris *et al.* 2020). In a series of elegant studies using the methods cited in these papers, a detailed map of the events that follow wound induction and lead to blastema formation have been identified. Some of the key events include the induction of calcium signaling waves, the generation of reactive oxygen species, the triggering of MAP kinase signaling, and the activation of transcriptional targets required for cell proliferation. The final steps are to specify the fate of the regenerating tissue and to accurately pattern it (Worley *et al.* 2012; Worley and Hariharan 2021).

In general, imaginal disc fragments regenerate themselves in 2 distinct ways. A blastema will either give rise to the missing portion of the disc or it will duplicate the transplanted fragment. Whether the blastema regenerates missing structures or duplicates existing portions of the disc depends upon the type of imaginal disc and/or the size of the fragment that was transplanted into the host larva (Bryant 1971; Schubiger 1971). In a limited number of cases, cells of the blastema will adopt the incorrect fate and the resulting tissue (after metamorphosis) will be a mosaic of at least 2 adult tissues. Why do regenerating cells sometimes adopt the wrong fate? Gerold Schubiger proposed that changes to the character of the cell cycle preceded transdetermination and that such changes are induced by mis-regulation of epigenetic factors (Sustar and Schubiger 2005). This model is supported by the eye to wing transformation that is seen in Pc RNAi induced knockdowns (Zhu *et al.* 2018). PcG and Trithorax Group factors control the expression of hundreds of developmentally regulated genes including those that encode imaginal disc selector genes. Thus, each transdetermination event that has been recorded could potentially be explained by changes in the expression of epigenetic factors and selector genes within the blastema. Based on genetic and molecular studies (Kim *et al.* 1996; Simmonds *et al.* 1998; Zhu *et al.* 2018; Ahmad and Spens 2019), the transdetermination of the eye into a wing, for example, would be predicted to be caused by the mis-regulation of *vg* expression within the blastema.

Growth of the eye-antennal disc

Reflecting the differing sizes of adult appendages, each imaginal disc is comprised of dramatically distinct numbers of cells (Fig. 10). For example, while the late third larval instar haltere disc contains 7,500–10,000 cells, the wing disc has between 43,000 and 52,000 cells (Garcia-Bellido *et al.* 1970; Garcia-Bellido and Merriam 1971a; Morata and Garcia-Bellido 1976; Steiner 1976). Likewise, while the third thoracic leg has 17,000–21,000 cells, the eye-antennal disc is estimated to contain approximately 44,000 cells (Steiner 1975; Martin 1982). The eye portion is comprised of roughly 25,000 cells while the antennal disc contains nearly 19,000 cells (Becker 1957). Some studies utilized indirect methods such as the growth curves of imaginal discs and volume measurements to approximate the number of cells per imaginal disc. These estimations turned out to be remarkably accurate and hue closely to numbers that were obtained from directly counting cells in histological preparations or through use of a hemacytometer. Since the growth of imaginal discs is complete at or around the larval/pupal transition, it was of interest to understand how imaginal discs could achieve vastly different sizes despite having to do so within an identical time frame. Early studies of imaginal discs identified 3 general features that are important the differences in adult appendage size.

First, the number of founder cells that are allocated for each imaginal disc varies considerably (Fig. 10). The most reliable estimates of founder cell numbers come from direct counts of cells within imaginal discs of newly hatched first instar larvae (Mandaravally Madhavan and Schneiderman 1977). At this stage the eye-antennal disc is comprised of approximately 77 cells of which 42 will give rise to the eye disc and the remaining 35 will produce the antennal disc. In contrast, about 20 founder cells form the haltere, 38 produce the wing, and between 36 and 45 cells are set aside for each of the 3 types of legs. Although the differences in founder cell numbers can only be directly visualized at the time of allocation (late embryogenesis) an analysis of the size of X-ray induced mitotic clone size suggested that these differences are already encoded within the blastoderm stage embryo (Wieschaus and Gehring 1976). This provides further evidence that the imaginal disc fates are established early in development.

Second, after proliferation ceases in most embryonic tissues (the nervous system being a major exception) each imaginal disc reinitiates cell division at different larval stages (Fig. 10). The eye is the first tissue to reinitiate proliferation at 13–15 h after the first instar larva hatches from the eggshell. The wing and haltere discs follow and restart growth in successive waves with the former reinitiating cell division at 15–17 h and the latter at 18–20 h. Lastly, leg, genital, and antennal discs all resume mitotic divisions simultaneously at 24–26 h (Mandaravally Madhavan and Schneiderman 1977). It is interesting that the eye and antennal portions of the disc re-establish cell proliferation at distinct times

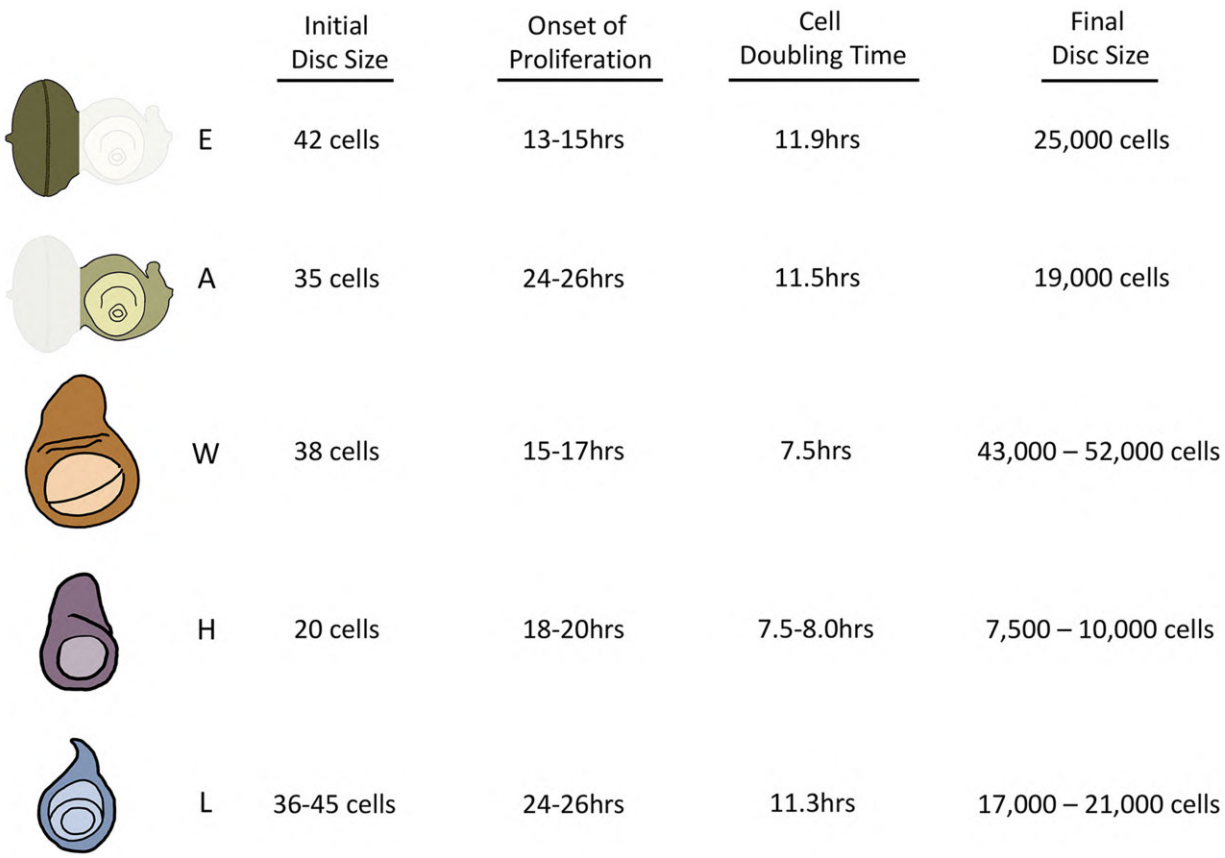

Fig. 10. Growth characteristics for the imaginal discs of Drosophila. Each external appendage (and hence the imaginal disc from which it is derived) has a distinct final size. Several factors influence how the final size of each adult structure is achieved. These include starting with unique number of cells, initiating proliferation at different times during the first larval instar, and doubling in size at distinct rates. E, eye; A, antenna; W, wing; H, haltere; L, leg.

in larval development. It is further evidence that their fate is determined early in embryogenesis. Since the eye-antennal disc arises from more than just 2 embryonic head segments, is it possible that the onset of proliferation is even more regionalized. A high-resolution proliferation map may reveal subtleties in the spatial patterns of cell proliferation that were not possible to observe with older histological methods.

Finally, and not surprisingly, the time that it takes for cell numbers to double also varies across the imaginal discs (Fig. 10). The number of cells within the wing disc doubles almost every 8.5–10.6 h. This is substantially faster than the eye-antennal disc which itself doubles in size every 11.5–11.9 h. Interestingly, in this respect the eye and antennal portions of the disc have nearly identical doubling rates (11.9 and 11.5 h, respectively) (Patterson 1929; Bryant 1970; Garcia-Bellido and Merriam 1971a, 1971b; Postlethwait and Schneiderman 1971; Haynie 1975; Morata and Garcia-Bellido 1976; Steiner 1976; Mandaravally Madhavan and Schneiderman 1977). Since each part of the eye-antennal disc contributes a distinct number of cells to the adult head, the doubling rate of the disc is likely to be more complicated. In other words, the eye-antennal disc is not a single unit but rather it is a collection of several tissues—each growing independently side by side. The inequity in starting cell numbers, the unique starting time for re-establishing mitotic divisions, and the distinct rate of proliferation allows for the 2 eye-antennal discs to produce an adult head that is of the right size by the time discs must undergo metamorphosis.

The final size of the compound eye is also controlled, in part, by 2 waves of mitosis. These waves were first identified when eye-antennal discs were analyzed for the incorporation of radiolabeled thymidine into actively dividing cells (Ready *et al.* 1976; Campos-Ortega and Hofbauer 1977). Using the same technique, 2 mitotic waves were also seen in the eye of the developing moth suggesting a common evolutionary mechanism for generating the correct number of cells within the compound eyes of insects (Egelhaaf *et al.* 1975). The first mitotic wave is a broad band of mitosis that lies ahead of the morphogenetic furrow and generates a large pool of cells. The morphogenetic furrow then takes a significant fraction of these cells and organizes them into the roughly 750 individual units that make up the adult eye (Clayton 1954a, 1954b; Ready *et al.* 1976). As such, the first mitotic wave, in essence, sets the number of ommatidia that will be generated.

The mature ommatidium is an assembly of 8 photoreceptors and 12 non-neuronal accessory cells (Dietrich 1909; Wolken *et al.* 1957; Waddington and Perry 1960). However, the furrow only brings 5 cells into each unit (Ready *et al.* 1976; Tomlinson and Ready 1987a, 1987b). Where do the remaining 15 cells come from? All cells that are not initially incorporated into the rudimentary ommatidia will undergo one final round of cell division. These are seen as a tight band of mitoses a few rows behind the morphogenetic furrow. This is referred to as the second mitotic wave. It produces the remaining photoreceptor neurons, cone and pigment accessory cells, as well as cells of the bristle complex (Ready *et al.* 1976; Tomlinson and Ready 1987a, 1987b; Wolff and Ready 1991a). Together these 2 waves of mitosis produce all cells necessary to generate a compound eye containing 750 fully assembled unit eyes (Clayton 1954a, 1954b; Ready *et al.* 1976).

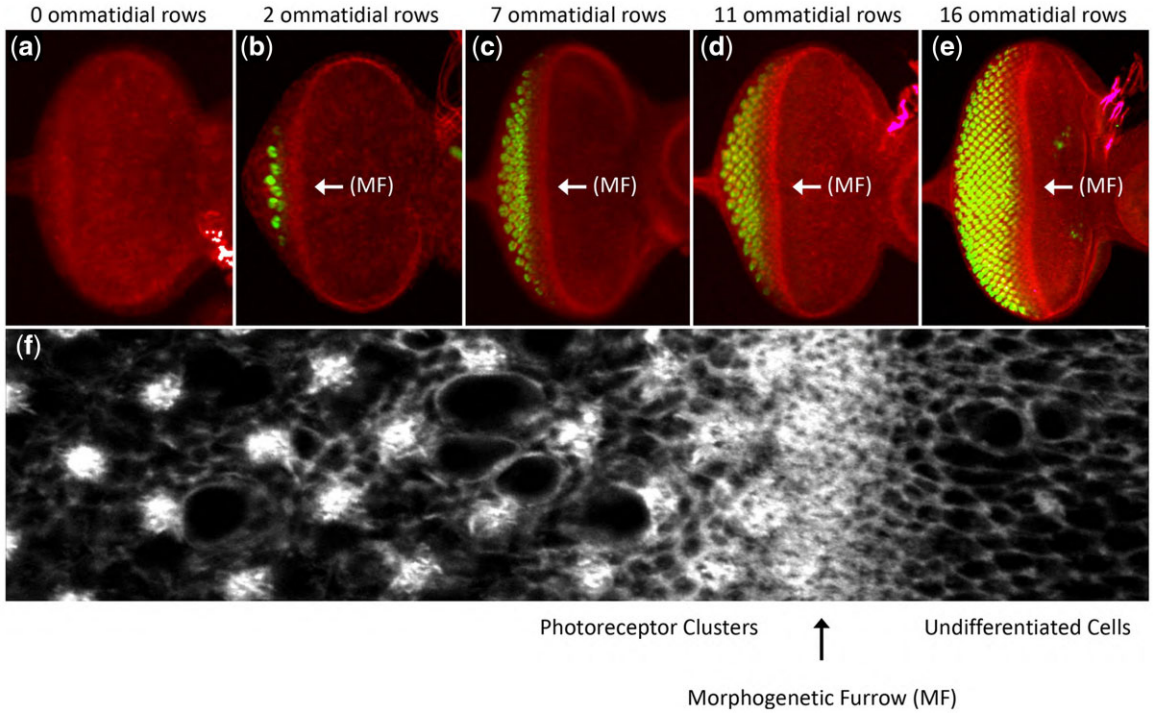

Fig. 11. The morphogenetic furrow patterns the eye-antennal disc. a–f) Light microscope images of third larval instar eye-antennal discs. a) At the L2/L3 transition, there are no signs of photoreceptor development. b–e) As development proceeds, the morphogenetic furrow initiates patterning at the posterior margin of the disc. It traverses in the anterior direction until it reaches the border of the eye and antennal fields. As it moves across the eye field, the sea of undifferentiated cells is transformed into orderly rows of periodically spaced unit eyes. f) A higher magnification view of a late third larval instar eye-antennal disc showing the transformation of an undifferentiated field into an ordered array.

Patterning the eye

The drawing of the eye-antennal disc in August Weismann's monograph of imaginal discs includes a feature that we know today as being the morphogenetic furrow. However, at the time, this groove in the epithelium, which runs across the dorsal-ventral axis, was thought to be a border that separates the eye from the antenna. Light microscope studies of eye-antennal discs from third instar larvae seemed to confirm this idea as cells on one (anterior) side of Weismann's "eye/antennal border" are unpatterned while periodically spaced clusters of cells reside on the other (posterior) side—these latter groupings are the future ommatidia (Krafka 1924; Chen 1929; Medvedev 1935; Steinberg 1943a, 1943b; Waddington and Perry 1960). A key revelation about how the eye field is patterned came when Donald Ready and Seymour Benzer observed that Weismann's border could be found at different positions along the anterior-posterior axis depending upon the point at which the disc was examined during development. At the beginning of the third larval instar, it is located toward the posterior end of the eye field while it could be found at more anterior positions later in development. The changing position of the "border" from posterior to anterior positions correlated with a shift in the ratio of unpatterned to patterned tissue. As the larva progresses through the third instar the number of ommatidia grows at the expense of the unpatterned field (Fig. 11a–f). Quickly realizing that the "border" is not a border at all but instead is a differentiating wave, Ready and Benzer dubbed it the morphogenetic furrow. The furrow initiates at the posterior margin and proceeds anteriorly until it reaches the true eye/antenna border (Fig. 11a–e) (Ready *et al.* 1976). Similar differentiating waves have been described for the development of mammalian molars, feather buds of birds, and somites of vertebrates (Gaunt 1961; Wessells 1965; Cooke 1975).

How does the morphogenetic furrow traverses across the disc? One model proposed that substances secreted from developing photoreceptors pushed the furrow across the eye field (Ready *et al.* 1976). In contrast, in their much over-looked paper on eye development, Richard Lebovitz and Donald Ready came to a very different conclusion. They transplanted a fragment of the eye-antennal disc that contained the antenna and the most anterior region of the eye into host larvae. After metamorphosis, these transplants developed into heads that, of course, contained derivatives of the antennal segment. However, astonishingly, it also contained a compound eye. This was quite surprising since the transplanted fragments did not contain either photoreceptor clusters or the morphogenetic furrow, both of which are required for retinal patterning. These data led the authors to conclude that factors lying ahead of the furrow were "pulling" it forward across the epithelium (Lebovitz and Ready 1986). As we will see below, this model turned out to be incorrect and the furrow is in fact pushed across the eye field.

However, as mentioned above, the ability of anterior eye fragments to produce compound eyes presaged the discovery of the retinal determination network. Interestingly, this gene regulatory network is not sufficient to restart eye development in these fragments. So how is repatterning of the eye field accomplished? Insights into this question came more than a decade later from a study of regenerating leg fragments. The authors asked how anterior fragments of the leg disc could regenerate or duplicate themselves without a source of Hedgehog (Hh) signaling from the posterior compartment (which was excised and discarded). This is a similar question to eye disc fragments. The answer to this question was surprising as a new source of Hh signaling, the overlying PE, was identified. Hh from the PE could stimulate proliferation and restart patterning of the blastema (Gibson and

Schubiger 1999). Hh, as well as Decapentaplegic (Dpp), signaling is also present within the PE of the eye-antennal disc (Cho *et al.* 2000; Gibson *et al.* 2002). As such, it is possible that these 2 pathways, which are essential for morphogenetic furrow initiation, could induce a new furrow at the blastema site of anterior eye disc fragments.

Resolution to the whether the furrow is pushed or pulled came from the discovery that the Hh morphogen is responsible for pushing the furrow across the eye field (Heberlein *et al.* 1993; Ma *et al.* 1993). Hh is produced within a band of developing photoreceptors that lie just posterior to the furrow. This short-range morphogen is captured by cells that lie within the furrow itself (Benlali *et al.* 2000; Corrigall *et al.* 2007). Activation of the Hh pathway within the furrow triggers the production and secretion of the long-range Dpp morphogen, which in turn, is trapped by the cells that lie ahead of the advancing furrow (Blackman *et al.* 1991; Heberlein *et al.* 1993; Ma *et al.* 1993; Pan and Rubin 1995; Borod and Heberlein 1998; Greenwood and Struhl 1999; Pappu *et al.* 2003). In response, cells ahead of the furrow activate expression of select retinal determination network genes and enter G1 arrest as they await the decision to become part of the rudimentary ommatidium or proceed into the second mitotic wave (Penton *et al.* 1997; Horsfield *et al.* 1998; Chen *et al.* 1999; Curtiss and Mlodzik 2000). Mutations in either *hh* or *dpp* prevent or slow the furrow from advancing across the eye field (Mohler 1988; Lee *et al.* 1992; Heberlein *et al.* 1993; Ma *et al.* 1993; Burke and Basler 1996).

Both Hh and Dpp signaling have also been implicated in initiating pattern formation at the posterior edge of the disc. At the L2/L3 transition, *hh* is expressed at the point where the midline of the disc intersects the posterior margin—this region is referred to as the "firing point" (Dominguez and Hafen 1997; Borod and Heberlein 1998). *dpp* is transcribed on either side of the firing point and extends along most of the posterior-lateral margins (Masucci *et al.* 1990). Hh signaling at the margin is required to maintain *dpp* expression and initiate retinal differentiation (Borod and Heberlein 1998). If either Hh or Dpp signaling is eliminated from the margins early in development, then the initiation of the furrow is abrogated (Heberlein *et al.* 1993; Ma *et al.* 1993; Chanut and Heberlein 1997a, 1997b; Hazelett *et al.* 1998). Consistent with the idea that Hh and Dpp signaling can, on their own, initiate eye development, the ectopic activation of either pathway ahead of the endogenous morphogenetic furrow is sufficient to induce ectopic photoreceptor development and an ectopic morphogenetic furrow (Chanut and Heberlein 1995; Heberlein *et al.* 1995; Ma and Moses 1995; Pan and Rubin 1995; Dominguez and Hafen 1997; Pignoni and Zipursky 1997). This provides additional support for a model in which Hh and Dpp from the PE could force regenerating cells along the edge of a disc fragment into producing a compound eye de novo and without a neural template.

Several other cascades including the Notch, EGF Receptor, and JAK/STAT signaling pathways are also required for the initiation of the furrow at the firing point and its re-initiation along the posterior and lateral margins. Like Hh and Dpp signaling, reductions in these 3 additional pathways prevents the furrow from initiating while ectopic activation at the lateral margins results in the formation of ectopic differentiating waves (Kumar and Moses 2001b; Ekas *et al.* 2006; Tsai *et al.* 2007). Once the fate of the eye is specified by the retinal determination network, the above signal transduction pathways then act to pattern the retinal epithelium by initiating the morphogenetic furrow and helping it traverse across the epithelium. These 2 processes (specification and patterning) appear to be linked to each other as the retinal determination network and the aforementioned signaling pathways and appear to cross-regulate one another (Hazelett *et al.* 1998; Chen *et al.* 1999; Kumar and Moses 2001a; Kango-Singh *et al.* 2003; Pappu *et al.* 2003, 2005; Li *et al.* 2013; Baker *et al.* 2018; Weasner and Kumar 2022).

The eye has the curious feature that it is patterned by the morphogenetic furrow while it continues to grow. As such the rate of patterning must be synchronized with the rate of growth. If the furrow moves too fast or too slow then the eye will be smaller than it should be (albeit for different mechanistic reasons). The adult compound eye has between 32 and 34 vertical columns of ommatidia. The first measurements of how quickly the furrow moves across the eye disc came from 2 studies which indicated that it lays down a row ever 1.5–2 h. In one study, radiolabeled thymidine was injected into larvae at different times and adult retinas were later assayed for how many columns of ommatidia contained the radiolabeled nucleotide (Campos-Ortega and Hofbauer 1977). The other approach examined which and how many adult columns of ommatidia contained R7 cells after timed pulses of Sevenless (Sev) were delivered to *sev* mutant larvae (Basler and Hafen 1989). In contrast, direct counting of ommatidial rows in carefully timed and aged wild-type larvae later showed that the velocity of the furrow as it moves across the entire eye field encompasses a large dynamic range. The first few rows of ommatidia are generated at a fast rate of 35 min/row. As the furrow reaches the middle of the field, it slows considerably and produces new ommatidial columns at the slow-poke pace of a column every 150 min. The furrow then speeds up again and produces the last several rows at an approximate rate of 65–75 min/row. These changes in velocity are necessary for patterning to keep up with cell proliferation and for all ommatidia to be generated by the time the eye-antennal discs fuse together during the early hours of pupal development (Spratford and Kumar 2013).

The shifting rate at which the furrow traverses the eye field implies that molecular mechanisms exist to speed the furrow up when necessary and to slow it down when needed. A pair of studies have identified molecular brakes that appear to slow the furrow. If the nuclear hormone receptor Ultraspiracles (Usp) or the helix-loop-helix transcription factor Extramacrochaetae (Emc) are removed from the developing eye, then the furrow accelerates (Brown *et al.* 1995; Dominguez *et al.* 1996; Zelhof *et al.* 1997; Methot and Basler 2001). Emc appears to regulate the processing of Cubitus interruptus (Ci), which is the singular transcription factor within the Hh signaling pathway (Dominguez *et al.* 1996; Methot and Basler 2001). While its expression is not affected by the absence of Emc, the balance between the activating and repressing forms of Ci are shifted dramatically toward the activating version (Spratford and Kumar 2013). As such, Emc regulates the pace at which the furrow patterns the eye field by maintaining just the right level of Hh signaling that is appropriate for each position along the anterior-posterior axis of the eye field (Spratford and Kumar 2013, 2014). Undoubtedly additional molecular brakes and accelerators are out there to be identified.

The furrow initiates from only one spot along the posterior margin—the firing point (Ready *et al.* 1976). As similar phenomena have been observed in other systems it raised the question of how the specificity of pattern initiation is achieved. Initiating cellular differentiation from a single point is essential for ensuring the regularity of the pattern itself. For example, the compound eye is so precise in its form that it has been affectionately called a neurocrystalline lattice. If, on the other hand, patterning is initiated from several points then the region where the differentiating

waves crash into each other might be expected to be disorganized. This is precisely what happens when Wingless (Wg) signaling is compromised. Within the developing eye, *wg* is expressed along the dorsal and ventral margins where it appears to block the initiation of ectopic differentiating waves. In the absence of Wg signaling ectopic morphogenetic furrows appear to emanate from both dorsal and ventral margins. The resulting eyes are small, rough, and disorganized (Ma and Moses 1995; Treisman and Rubin 1995). Wg signaling, of course, does not function in a vacuum and so it would not be surprising if disruptions to other pathways/genes also result in the induction of ectopic differentiating waves. Indeed, additional morphogenetic furrows are launched from both the dorsal and ventral margins when *emc* expression is lost. It appears that Emc functions (in some contexts) upstream to regulate *wg* expression for the latter is lost at the dorsal margin but remains robustly expressed at the ventral margin when *emc* is removed from the eye disc (Spratford and Kumar 2013).

The distinct regulatory relationships that exist between Emc and Wg within a single tissue hint at an exciting feature—that the dorsal and ventral halves of the eye might originate from different precursor cells and fuse together during embryogenesis before the expression of any known molecular markers can identify the 2 individual units. The dual origin of the eye disc (if correct) would be consistent with a recent study which described the dorsal and ventral compartments of the wing disc as originating from distinct regions of the developing embryo (Requena *et al.* 2017). If the eye does in fact have a dual origin, it could provide mechanistic clarity to how the adult compound eye of many insects becomes separated by head cuticle, antennae, and head shields during development.

The compound eye of beetles within the Gyrinidae family (also called whirligig beetles) are divided into dorsal and ventral compartments by a strip of head cuticle that runs along the midline of the eye. These beetles swim along the surface of the water with the water line being at the level of the head cuticle. As the beetle swims along the water, the ventral eye is submerged while the dorsal eye lies above the water line. The lenes of the 2 eyes have different refractive indices to allow the beetle to see objects in the air and under water simultaneously and clearly (Blagodatski *et al.* 2014). While substantial data supporting a dual origin for the eye does not yet exist, fragmentary evidence for this idea can be found scattered in the literature. In one instance, the dorsal half of the eye was proposed to develop first with growth of the ventral half following (Won *et al.* 2015). Other studies, when taken together, could also support a model in which the dorsal and ventral halves develop independently of each other. One report seemed to confirm that the dorsal originates first (Baker *et al.* 2018) while a second study supported an alternate model that the ventral eye is the first to initiate its development (Singh and Choi 2003).

Ommatidial assembly

The development of the ommatidium is akin to an assembly line in the sense that the various cell types are added and specified within the unit eye in a sequential and synchronized order (Fig. 12). Each unit eye consists of 8 photoreceptors, 4 lens secreting cone cells, and a set of pigment cells that optically insulate one unit eye from the other. Several adjoining unit eyes also share a 4-celled mechanosensory bristle complex (Dietrich 1909; Johannsen 1924; Krafka 1924; Clayton 1954a, 1954b; Waddington 1962; Ready *et al.* 1976). The bristles are best seen in scanning electron micrographs of the external surface while the photoreceptors are best visualized in sections of adult eyes (Ready *et al.* 1976). The cone and pigment cells are best observed in sections of mid-pupal staged retinas (Cagan and Ready 1989a; Wolff and Ready 1991b).

The photoreceptors are the first cells of the ommatidium to have their fate specified. These cells are incorporated into the ommatidium in 2 successive phases. The morphogenetic furrow will first bring 5 cells together to form what is called the precluster—this is the rudiment of the future ommatidium. One cell of the precluster will adopt the fate of the R8 photoreceptor neuron. A pair of adjacent cells will then be specified as the R2/5 photoreceptors. Finally, the last 2 cells of the precluster will adopt the identity of the R3/4 pair. The last 3 photoreceptors (R1/6/7) are generated by the second mitotic wave and added to the growing unit eye with the R1/6 pair being added first followed by the R7 neuron (Fig. 12) (Ready *et al.* 1976; Tomlinson and Ready 1987a, 1987b).

Within the ommatidium, the photoreceptors are organized into an asymmetrical trapezoid with each neuron occupying a specific stereotyped position within the unit eye (Fig. 13, left panel) (Dietrich 1909). One major difference between individual unit eyes is that the photoreceptors in ommatidia within the dorsal half of the eye take on a chiral form that is the mirror image of those in the ventral portion. Similar symmetries exist also between right and left eyes (Fig. 13, right panel). The final orientation and chirality of the ommatidia result from the R3/4 precursors adopting different identities and positions with the ommatidium, the choice to rotate either clockwise or counterclockwise, and 2 steps of 45° rotations (Ready *et al.* 1976; Choi and Benzer 1994; Zheng *et al.* 1995; Tomlinson and Struhl 1999).

The cone cells, which are also generated by the second mitotic wave, are the last cells to be added to the ommatidium during larval life (Ready *et al.* 1976; Tomlinson and Ready 1987a, 1987b; Wolff and Ready 1991a). Assembly of the ommatidium is completed during pupal development when all 3 classes of pigment cells (primary, secondary, and tertiary) and the bristle complex are recruited into each growing unit eye (Cagan and Ready 1989a). Patterning of the entire eye is finished when a synchronous wave of cell death across the retina eliminates excess cells from each ommatidium and a separate ring of apoptosis around the periphery of the retina removes stunted ommatidia thereby creating a smooth transition from ommatidia to head epidermis (Wolff and Ready 1991b; Tomlinson 2003; Lim and Tomlinson 2006; Kumar *et al.* 2015).

Flies harboring mutations in genes that control ommatidial assembly often have compound eyes that have a smaller than normal number of unit eyes. In some mutants, the ommatidia are even fully absent. For example, *atonal (ato)* mutants lack ommatidia altogether while *Bar (B)*, *Drop* (*Dr*), *atonal* (*ato*), and *lozenge (lz)* mutant flies have dramatically reduced numbers of unit eyes (Tice 1914; Zeleny and Mattoon 1915; Chen 1929; Krivshenko 1954; Jarman *et al.* 1994). Early researchers discovered that the number of ommatidial facets in *Bar* and *lz* mutants could be altered by the application of higher and lower than normal temperatures. In some cases, the number of ommatidia would be further depressed while in other instances, the number of unit eyes would increase substantially. These studies were very interesting in that the administration of different temperatures would only affect ommatidial numbers if applied during the third larval instar (Hoge 1915; Seyster 1919; Krafka 1920a, 1920b, 1920c; Metz 1923; Hersh 1924a, 1924b; Driver 1926; Hersh 1930). As such, these researchers had essentially identified the time period at

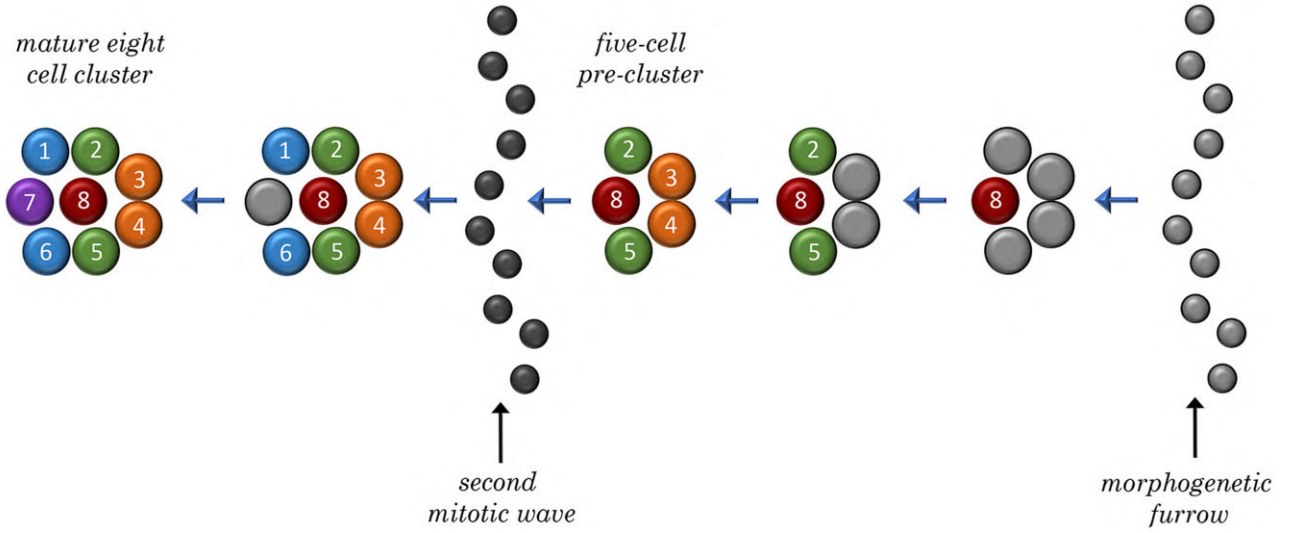

Fig. 12. The assembly line of photoreceptor development. The first mitotic wave produces many cells. The morphogenetic furrow sweeps up a fraction of those cells and organizes them into a periodic array of 5-cell preclusters. The first cell of the precluster to have its fate specified is the R8. This is followed by the specification of the R2/5 and the R3/4 pairs. All cells that are not incorporated into the precluster undergo one final round of cell division called the second mitotic wave. Three cells from the second mitotic wave are added to the growing cluster and are specified as the R1/6 and R7 photoreceptors. At this point in development, the ommatidium has a symmetrical arrangement.

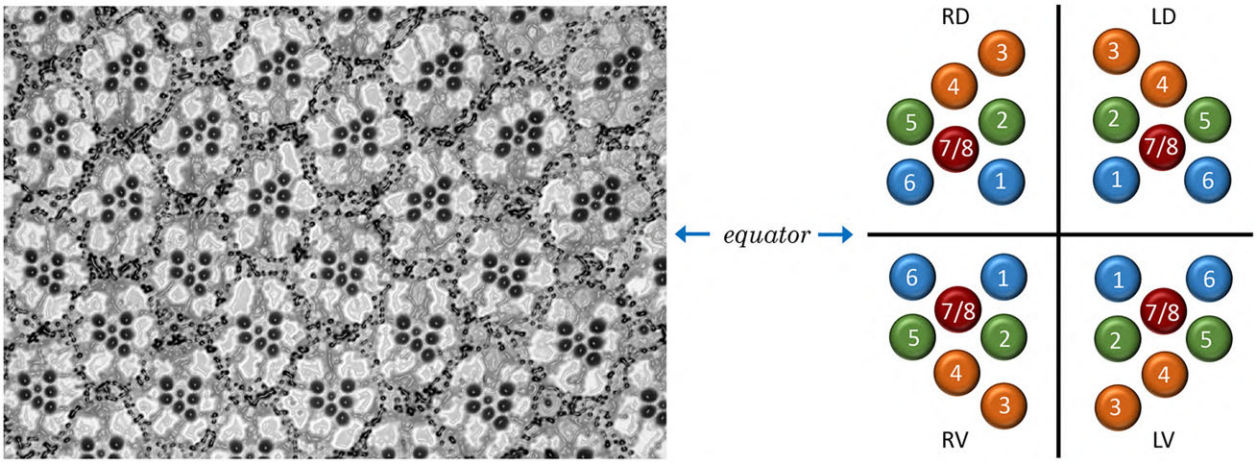

Fig. 13. Organization of the ommatidium. During larval development, the symmetrical arrangement of the ommatidium is broken and the photoreceptors are organized into the shape of an asymmetric trapezoid. The trapezoids in the dorsal half of the retina are mirror images of those that lie within the ventral half. These mirror images are the products of different chirality and rotation events. The point at which the dorsal and ventral compartments meet in the eye is called the equator. RD, right eye, dorsal compartment; LD, left eye, dorsal compartment; RV, right eye, ventral compartment; LV, left eye, ventral compartment.

which ommatidial assembly takes place. This time window was confirmed by direct histological analysis of developing eye-antennal discs. Clusters of ommatidia were seen in third larval instar discs, but not of second or first instar ones (Krafka 1924).

Over the subsequent decades, a large collection of mutants that affected the number of unit eyes and/or the overall structure of the compound eye were identified. While many mutants were like *ato*, *Bar*, *Dr*, and *lz*, in that the compound eyes had vastly fewer ommatidia, there were many instances in which the compound eye is of normal size but had a "roughened" or "glazed" appearance (Fig. 14). Histological preparations revealed the steps of ommatidial assembly that were affected by each mutant. As molecular tools became available, the underlying genes were cloned and their expression patterns within the ommatidia were determined (Kumar 2012). From these collective efforts, we have gained a more sophisticated understanding of how communication between cells contributes to cell fate choice.

One of the most celebrated results to come from studies of ommatidial assembly was the discovery of the Sevenless signaling pathway. Mutations in the *sev* gene were first identified by defects in phototaxis (Benzer 1967). Subsequent histological analysis of adult retinas determined that these visual deficits were due to the absence of the R7 photoreceptor (Harris *et al.* 1976). A developmental analysis of ommatidial assembly showed that in *sev* mutants, the R7 precursor instead adopts that fate of a non-neuronal cone cell (Tomlinson and Ready 1986, 1987a). Subsequent molecular analysis of the *sev* gene showed that it encoded a functional receptor tyrosine kinase (RTK) (Banerjee *et al.* 1987; Hafen *et al.* 1987; Basler and Hafen 1988; Bowtell *et al.* 1988; Simon *et al.* 1989). The identification of Sev as a RTK

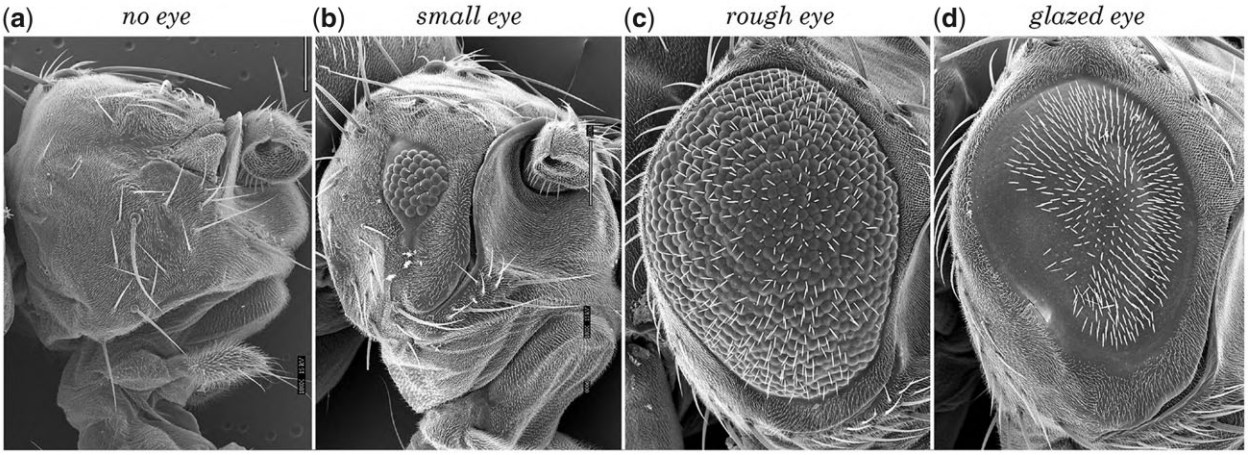

Fig. 14. The eye mutants of *Drosophila* reveal the roles of genes in development. a–d) SEM of adult *Drosophila* heads. a, b). Mutations that affect tissue specification, growth, patterning, and/or R8 cell often result in compound eyes that are missing or severely reduced in size. c, d) The loss of genes that affect later stages of ommatidial assembly (i.e. R2/R5, R3/4, R1/6, and R7 photoreceptors, cone cells, and pigment cells) result in large compound eyes that are "*roughened*" or "*glazed*" in appearance. Mutations that eliminate the bristle cells result in compound eyes that have a "*balding*" appearance (not shown).

confirmed what previous genetic studies suggested—that cell–cell communication via signal transduction and not cell lineage was responsible for cell fate choices within the ommatidium. It also triggered a frantic effort by Gerald Rubin, Seymour Benzer, and Ernst Hafen to elucidate the entire Sevenless pathway including cytoplasmic components and terminal transcription factors (Nagaraj and Banerjee 2004). Some of the most important findings were the determination that Ras and MAPK lie downstream of the Sev receptor and transduce information to terminal transcription factors (Simon *et al.* 1991; Fortini *et al.* 1992; Biggs *et al.* 1994; O'Neill *et al.* 1994; Brunner *et al.* 1994a, 1994b; Rebay and Rubin 1995; Kumar *et al.* 1998, 2003). These results paralleled findings that the Ras/MAPK cassette is shared by several RTKs in *Drosophila* including Torso and the EGF Receptor (Rogge *et al.* 1991; Doyle and Bishop 1993; Diaz-Benjumea and Hafen 1994; Brunner *et al.* 1994b). A key insight into R7 fate specification came from Larry Zipursky who demonstrated that R8 and R7 cells communicate with each other via the Bride of sevenless (Boss) ligand on the surface of the R8 and the Sev receptor on the R7 (Reinke and Zipursky 1988; Hart *et al.* 1990; Kramer *et al.* 1991; Van Vactor *et al.* 1991; Cagan *et al.* 1992). These studies combined the physical interactions of the 2 cells and a ligand-receptor complex to elucidate how cell–cell communication specified the last photoreceptor of the ommatidium.

The above studies of the R7 spurred interest in how the entire process of ommatidial assembly is initiated. The selection of the R8 cell is under the control of the Notch signaling pathway and the pro-neural transcription factor Ato (Cagan and Ready 1989b; Jarman *et al.* 1994; Baker and Zitron 1995; Jarman *et al.* 1995; Baker *et al.* 1996; Dokucu *et al.* 1996; Baker and Yu 1997; Powell *et al.* 2001). Once the R8 has been specified it secretes Spitz (Spi), a ligand for the EGF Receptor. This leads to the specification and recruitment of the R2/5 photoreceptor pair (Freeman 1994; Tio *et al.* 1994; Tio and Moses 1997). The EGF Receptor is then reiteratively used to sequentially recruit the R3/4 and R1/6 pairs (Freeman 1996; Dominguez *et al.* 1998; Kumar *et al.* 1998; Spencer *et al.* 1998). Studies of Notch and the EGF Receptor has led us to understand that R7 fate specification depends on more than just the Sevenless pathway. In fact, complex interplay between Sevenless, the EGF Receptor, and Notch signaling is important for not only specifying the R7 cell but also in distinguishing it from the other photoreceptors within the ommatidium (Cooper and Bray 2000; Tomlinson and Struhl 2001; Miller *et al.* 2008; Tomlinson *et al.* 2011; Mavromatakis and Tomlinson 2013).

The study of ommatidial assembly has contributed to our understanding that cell fate decisions are made not by individual regulators but rather by combinatorial codes of transcription factors. As noted, the R7 itself is controlled by at least 3 signaling pathways that each have multiple terminal transcription factors. One of the best characterized examples of a single regulatory enhancer being controlled by a combinatorial code of DNA binding proteins in the eye is the enhancer for the DPax2 gene. Mutations that disrupt this enhancer were identified by the roughening of the eye surface (Belgovsky 1937). Based on the shimmering color of the eye, the allele was named *sparkling* (*spa*). This enhancer is activated in and required for the development of the 4 lens-secreting cone cells (Fu and Noll 1997). Molecular dissection of the enhancer indicates that it is regulated by a combinatorial code of at least a dozen, if not more, independently acting transcription factors (Flores *et al.* 2000; Swanson *et al.* 2010, 2011). These studies also provided new insight into the rules that govern the internal organization of the enhancer (i.e. binding site number, orientation, position, spacing, and affinity for transcription factors).

Concluding remarks

In this review, I have endeavored to provide an accounting of the early history of studies of the eye-antennal disc. I hope that this article has brought a greater appreciation for what early *Drosophila* researchers discovered despite being armed with only what we now consider to be crude genetic tools. For all the limitation of the era in which they did their work, the early researchers that I have referenced in this Flybook chapter provided an astonishingly accurate view of how the eye-antennal disc develops. Today's researchers have the distinct advantage of having cutting edge light microscopy methods at their disposal to view development in real time, creative genetic tools to manipulate the fly, and sophisticated molecular/biochemical tools to manipulate the genome. As such, much like archeologists, we are in the unique position of being able to shine a bright light on the eye-antennal disc and illuminate its treasures as we strive to

understand the mysteries of development. The early papers referenced herein provide a roadmap for achieving this goal.

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We would like to thank all the early researchers of the eye-antennal disc who came before us and gave us their inspiring stories and discoveries. The literature of the eye-antennal disc over the last century is vast; as such, we apologize if we have inadvertently overlooked important contributions by others. And lastly, we would like to thank Bonnie M. Weasner for her comments and suggestions on this manuscript.

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Conflicts of interest

None declared.

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