

The Eye of *Drosophila* as a Model System for Studying Intracellular Signaling in Ontogenesis and Pathogenesis

V. L. Katanaev^{1,2*} and M. V. Kryuchkov¹

¹*Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia*

²*Département de Pharmacologie et Toxicologie, Université de Lausanne, Lausanne, Switzerland; E-mail: vladimir.katanaev@unil.ch*

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Abstract—Many human diseases are caused by malfunction of basic types of cellular activity such as proliferation, differentiation, apoptosis, cell polarization, and migration. In turn, these processes are associated with different routes of intracellular signal transduction. A number of model systems have been designed to study normal and abnormal cellular and molecular processes associated with pathogenesis. The developing eye of the fruit fly *Drosophila melanogaster* is one of these systems. The sequential development of compound eyes of this insect makes it possible to model human neurodegenerative diseases and mechanisms of carcinogenesis. In this paper we overview the program of the eye development in *Drosophila*, with emphasis on intracellular signaling pathways that regulate this complex process. We discuss in detail the roles of the Notch, Hedgehog, TGF β , Wnt, and receptor tyrosine kinase signaling pathways in *Drosophila* eye development and human pathology. We also briefly describe the modern methods of experimentation with this model organism to analyze the function of human pathogenic proteins.

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STRUCTURE AND FUNCTION OF THE DROSOPHILA EYE

The eye of the fruit fly *Drosophila melanogaster* is a complex structure formed by a specific structural unit — the ommatidium, the corneal lens of which has the form of a convex hexagon facet. The faceted eye of the fruit fly is formed by 700 to 800 ommatidia with a transverse size of about 15 μm ; the overall size of the compound eye is 400 μm [1]. Ommatidia have their own light-refracting (dioptric), photoinsulative, and photosensitive components (Fig. 1).

The light-refracting apparatus of an ommatidium is formed by the transparent corneal lens and the crystalline cone (Fig. 1). Analysis of the cornea with atomic force and electron microscopy has revealed that facets are covered with a network of transparent cuticular nipples with the height of 30 nm and width of about 250 nm [2, 3]. These nanostructures facilitate penetration of visible light with $\lambda = 320\text{--}700$ nm from the air into the denser medium of the eye [4, 5] and help cleanse the eye through the

water-repellent function (the “lotus leaf” effect) [5]. The corneal lens operates in conjunction with the directly adjacent crystalline cone. The latter is a transparent body formed by four cone cells.

The photoinsulative apparatus of an ommatidium consists of pigment cells containing pigment granules possessing light-shielding function. Pigment cells increase the sensitivity of ommatidia and insulate rhabdomeres (light-sensitive photoreceptor membranes, see below) from sideways illumination. Each ommatidium contains two primary pigment cells, while the secondary and the tertiary pigment cells are shared between two and three neighboring ommatidia, respectively: the secondary pigment cells are located along the edges of the hexagonal ommatidium, and tertiary — in its alternating vertices (Fig. 1).

The photoreceptor function of an ommatidium is provided by eight light-sensitive or reticulum cells (photoreceptors), each of which carries a rhabdomere — a dense, initially apical plasma membrane formation that is responsible for light detection and generation of nerve impulses. Each rhabdomere contains about 60,000 microvilli filled with the visual pigment and having a diameter of 50 nm. Rhabdomeres of the outer photoreceptors

* To whom correspondence should be addressed.

R1-R6 ensure formation of visual images and penetrate the entire thickness of the retina. These rhabdomeres contain the protein opsin 1 (Rh1) capable of absorbing light in the visible range (absorption maximum – 478 nm). Between the outer rhabdomeres there are rhabdomeres of the central photoreceptors R7 and R8, each occupying half the thickness of the retina (Fig. 1). Opsin expression in these cells is strictly coordinated. On the basis of their photo-reflecting properties, two types of ommatidia are distinguished: pale and yellow [6]. Yellow ommatidia represent 65% of the total number of ommatidia and contain UV-sensitive R7-cells (absorption maximum – 375 nm) and R8-cells with absorption in the green spectral region (absorption maximum – 508 nm). R7 expresses opsin 4, and R8 expresses opsin 6. Pale ommatidia contain UV-sensitive cells R7 (absorption maximum – 345 nm) and R8 sensitive in the blue range (absorption maximum – 437 nm). R7 of the pale ommatidia express opsin 3, and the R8 cell expresses opsin 5 [7, 8]. There is also a specialized class of ommatidia along the dorsal (upper) edge of the eye (dorsal rim) that are sensitive to polarized light, and opsin 3 is present in both R7 and R8 cells of these ommatidia [9].

In addition to the visual function, the insect eye also has a tactile capacity due to the presence of mechanosensitive bristles arranged in alternating hexagon vertices of the ommatidia (Fig. 1) and outstanding by 15-20 μm above the surface of the eye.

STAGES OF EYE DEVELOPMENT IN DROSOPHILA

The development of flies occurs with complete metamorphosis (the holometabolic life cycle). Larvae have imaginal discs – groups of cells from which structures of the imago (adult) are formed at the pupal stage while larval tissues are destroyed. The eye, along with the antenna and the adjacent areas of the cuticle, is formed from the eye-antennal imaginal disc. This disc is formed from a part of the cellular blastoderm, consisting of approximately 20 cells, and is located in the dorsolateral ectoderm. In the early stages, the eye-antennal imaginal disc distinguishes itself from the surrounding tissue by expression of proteins Twin of eyeless (Toy) and Eyeless (Ey), homologs of the transcription factor Pax6, which in mammals is also expressed at the earliest stages of eye formation. Ectopic expression of Ey and Pax6 in flies can cause formation of eyes in new, unusual places [10, 11].

Up to the third instar larval stage the disc simply increases in size due to cell proliferation. Thus, by the end of the first instar larval stage the disc contains about 130 cells, while by the beginning of the third – about 10,000. This sequence of divisions is called “the first mitotic wave” [1]. By the third instar larval stage the eye-antennal imaginal disc is a monolayer of actively dividing cells

of columnar epithelium. At about 70 h before pupariation (at 20°C) sequential differentiation of eye cells begins. The first cells to stop division are those located at the back (posterior) position of the disc. In these cells, a change of expression profile takes place, and they begin to transmit a signal to neighboring cells, which, in turn, leads to cessation of division in them. Thus, the process sequentially involves more new cells. The division arrest is accompanied by changes in the cell shape, which result from constriction of the actin cytoskeleton, and coincides with the transition of cells to the G1 phase of the cell cycle. Under the microscope one can see how the front called morphogenetic furrow (MF) sweeps from the posterior to the anterior side of the imaginal disc (Fig. 2). MF moves through the disc until it reaches its anterior in about 2 days [1]. The initiation of MF is divided into birth and reincarnation. The birth is the appearance of the first group of cells that have passed to the G1 stage of the cell cycle. These cells trigger the whole cascade of promotion of MF. However, the imaginal disc is a rounded structure and, until MF reaches the middle of the disc, more and more rows of cells on each side are being continuously included. In each such row the furrow should be re-initiated. This process is called reincarnation.

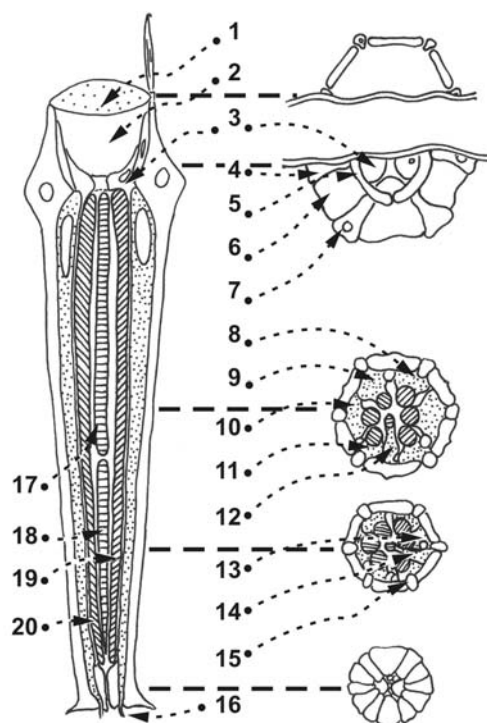


Fig. 1. Structure of the ommatidium in longitudinal section (left) and cross-sections at several levels of the retinal depth (right). 1) Corneal lens; 2) crystalline cone; 3) cone cell; 4) tertiary pigment cells; 5) primary pigment cell; 6) secondary pigment cell; 7) bristle; 8-12) R3-R7 photoreceptor cells, respectively; 13) R2 photoreceptor cell; 14) R8 photoreceptor cell; 15) R1 photoreceptor cell; 16) axons; 17, 18) R7 and R8 cell rhabdomeres, respectively; 19, 20) external photoreceptor rhabdomeres.

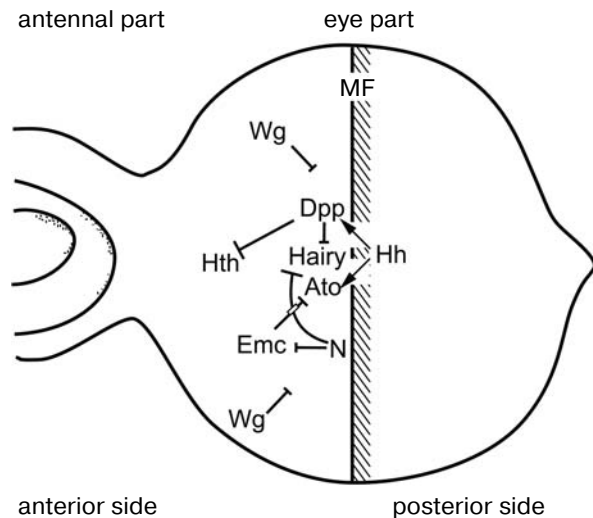


Fig. 2. Morphogenetic furrow (MF) in the eye-antennal disc of a third instar larva and factors that govern its progression. Originating at the posterior pole, MF moves towards the anterior. Cells “behind” (on a posterior side of) MF are at different stages of differentiation; the cells ahead of MF are undifferentiated. Hedgehog (Hh) is synthesized by differentiating cells at the front of MF, stimulating expression of the proneural factor Atonal (Ato). Another target of Hh is Decapentaplegic (Dpp), which diffuses farther than Hh beyond MF into the undifferentiated region to prepare cells for neuronal differentiation through repression of Homothorax (Hth). Notch (N) is activated in the MF area through stimulated expression of its ligand Delta under the control of redundant Hh- and Dpp-dependent signaling cascades and promotes neuronal differentiation through suppression of repressors Hairy and Extra macrochaetae (Emc). Wingless (Wg) diffuses from the head capsule to play a limiting role preventing initiation of ectopic MF.

As described above, MF is a zone of cell division arrest. Immediately behind MF sequential differentiation begins (Fig. 3). First, individual cells choosing the path of neuronal differentiation appear within the undifferentiated tissue at regular distances from each other. These are

the future photoreceptors R8. They recruit four neighboring cells, which also become photoreceptors: R2, R3, R4, and R5. Next R1 and R6 cells join the premature ommatidium. The last photoreceptor cell to be specified is R7 [1]. As MF moves in space, behind it (i.e. on the posterior side from MF) one can see the succession of the stages of compound eye formation described above. For convenience, researchers identify each stage of formation of ommatidia behind MF as a “row”. In the front row R8-cells start to specialize, while the second row contains the R8-cells which specialized 90 min earlier. Thus, while the precluster of five photoreceptor cells is forming in the second row, R1 and R6 are specializing in the fifth row, and R7 – in the seventh. By the end of the larval stage about 26 rows of ommatidia are formed. The remaining ones are completed within the first 10 h of pupariation [1].

Between the third and fifth rows of ommatidia, when R8 cells and R2-R5 cells are specialized and together constitute the ommatidial precluster, the undifferentiated cells surrounding the photoreceptors divide again. This division is called the second mitotic wave and serves to increase the number of cells available for subsequent phases of recruitment to the ommatidia [1].

Rhabdomeres of the adult ommatidium in a cross-section form an irregular trapezoid, that is, a figure that has chirality (Fig. 1). From the anterior to the posterior edge of the eye runs the equator – an imaginary line drawn between mirroring ommatidia of the upper and lower halves of the eye (Fig. 4). Ommatidial chirality is defined by photoreceptors R3 and R4, and is formed in the imaginal eye-antennal disc shortly after MF passes, when there are five cells in the ommatidial precluster, and is accompanied by rotation of the preclusters by 90° (see below, section “Frizzled/PCP-Signaling Cascade in Regulation of Ommatidial Polarity in *Drosophila*” of chapter “Non-canonical Frizzled/PCP-Signaling Cascade”).

The last photoreceptor cell to differentiate is R7, and it is different from the rest of the photoreceptors so that

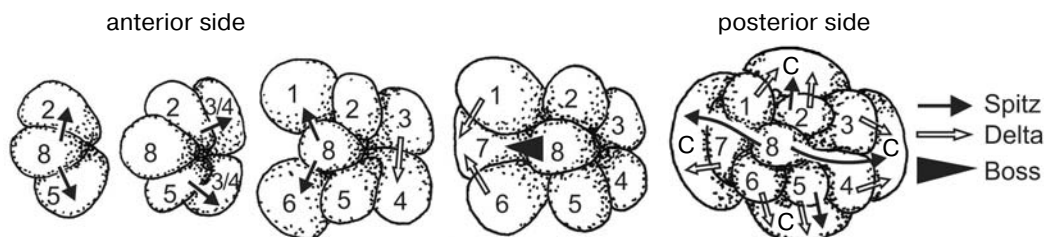


Fig. 3. Sequence of cell differentiation in ommatidia. Different stages of development of ommatidial preclusters are displayed, from the earliest (on the left, near MF) to mature. The first to emerge R8-photoreceptor recruits photoreceptors R2 and R5, sending the EGFR-activating Spitz-ligand (black arrows), which is later on used for differentiation of photoreceptors R3/R4 and cone (C) cells. R7 photoreceptor receives a double signal: activation of the Notch-receptor by Delta-ligands (open arrows) from R1 and R6 cells, and activation of the Sevenless-receptor by the Boss-ligand from the R8-photoreceptor (black arrowheads). Cone cells also receive a double signal: Spitz and Delta. The Notch-signaling cascade is additionally involved in specification of the R4-photoreceptor from the R3/R4-pair of initially equivalent progenitor cells (shown in the third ommatidial precluster).

for proper specialization it requires the simultaneous activation of two types of signaling cascades [12] (see section “The Notch-Cascade in Development of Photoreceptor R7” of chapter “The Notch-Signaling Cascade” and chapter “The Receptor Tyrosine Kinase-Signaling Cascade”): the Delta signal from R1 and R6 cells on one hand and the Boss signal from the R8 cell on the other (Fig. 3).

When differentiation of photoreceptors is over, non-neuronal cells start to specialize. At first cone cells are formed. After them the primary, secondary, and tertiary pigment cells emerge. The last to differentiate are mechanosensitive bristles, one for every three ommatidia.

After completion of cell division, cells receive a signal from photoreceptors that controls their survival through activation of the receptor-tyrosine kinase pathway (see section “The RTK-Signaling Cascade in *Drosophila* Photoreceptor Recruitment” of chapter “The Receptor Tyrosine Kinase-Signaling Cascade”). Cells that have not received enough of this signal die. This is the first wave of apoptosis, in which one cell dies among every 3–5 ommatidia. A few days later, during late stages of differentiation, the second wave of apoptosis takes place. At this time in each ommatidium 2–3 cells die, and the survival signal comes from cone and primary pigment cells. Surviving cells become secondary and tertiary pigment cells, or divide, producing mechanosensitive bristles [1].

A bristle consists of four cells, which are formed by three divisions of a single cell precursor. Formation of bristles of the eye has not been sufficiently investigated, but it apparently occurs along the same rules as formation of mechanosensitive bristles located on the fly thorax and wings. The basis of bristle formation is the asymmetry of divisions of cell precursors. During the first cell division, the precursor produces the anterior and posterior daughters, which differ in their content (in particular in the presence or absence of the protein Numb). The anterior daughter, which inherits Numb, asymmetrically divides two more times with the formation of inner cells of sensory bristles (a neuron, a glial cell and a supporting cell of the neuron membrane (sheath cell)). The posterior cell not inheriting Numb divides once asymmetrically to form the outer cell components of the bristle: the hair and the shaft [13].

As will be seen below, each of the stages of eye development is strictly regulated by intracellular signaling systems. Long-term studies of the *Drosophila* visual organ have produced extensive knowledge explaining which signaling pathways are responsible for which stages of morphogenesis, and which kind of morphological and histological disorders occur in the eye from perturbations of the signaling mechanisms. Given the high degree of homology and interchangeability of components of signaling pathways between insects and mammals, all of this makes the developing eye of *Drosophila* an ideal model system for studying the mechanisms of human signaling

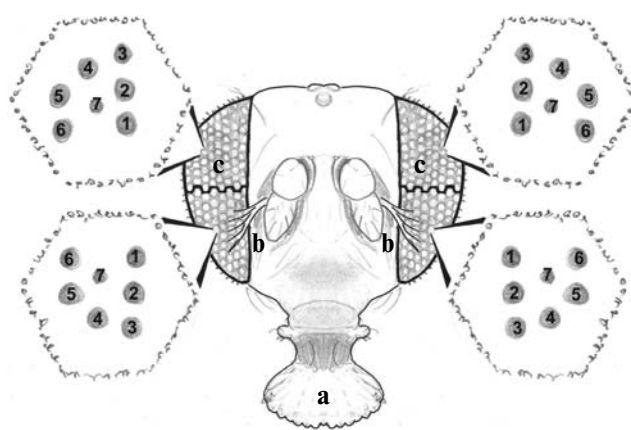


Fig. 4. Chirality and orientation of ommatidia in the eye of *Drosophila*. The head of *Drosophila* is shown “enface” with the oral apparatus (a), antennae (b), and eyes (c). Cross-sections of four ommatidia, one per each hemisphere of each eye (taken at the level of R7-photoreceptors), are schematically magnified. Numbers indicate rhabdomeres of photoreceptors R1–R7. The cross-sections reveal chirality in the arrangement of rhabdomeres of the six outer photoreceptors: photoreceptor R3 is “pushed” out from the group of remaining photoreceptors. The universality of orientation of the ommatidia is also obvious: they always point with their R3-photoreceptors in the polar direction (towards the head capsule). Chirality and orientation of ommatidia in the dorsal half of the eye are the mirror-reflected image of the ventral half; the eye has an imaginary line of reflection (the equator). In addition, chirality and orientation of the left eye ommatidia are mirror-reflected in the right eye.

proteins. The following sections describe in detail the signaling pathways involved both in the development of the fruit fly’s eyes and various human pathologies.

SIGNALING PATHWAYS THAT REGULATE EYE DEVELOPMENT IN DROSOPHILA

Signal transmission in the cell (intracellular signaling, cell signaling) is part of a complex system of communication that governs basic cellular processes and coordinates the activities of the cell. The ability of cells to respond properly to changes in their environment is the foundation of development, tissue regeneration, immune system, and homeostasis maintenance in general. The process of signal transmission involves a chain of biochemical reactions inside the cell, based either on protein–protein interactions and posttranslational modifications, or on stimulation/inhibition of the production of second messengers – low molecular weight intracellular substances which in turn regulate the activity of protein signal transmitters. Typically, the number of molecules involved in cell signaling increases with the progression to each following step of signal transmission. This process is called amplification of the signal. Thus, one talks about the signaling cascade that begins with a relatively weak

stimulus and causes a significant response. We should add that both positive and negative feedbacks are present in signaling cascades, and there is a considerable overlap of different signaling pathways. In this regard, the representation of intracellular signal transduction as a simple linear chain of reactions is a simplification, and more recently studies of signaling as a network of molecular interactions started to gain popularity [14, 15].

There are very few types of signal substances and their receptors, and thus types of signaling pathways that transmit them. For example, there are only five basic types of signaling pathways that are active during early embryogenesis [16]: (i) the Notch-signaling cascade; (ii) the Hedgehog-dependent signaling cascade; (iii) TGF β -cascades; (iv) Wnt/Frizzled-signaling cascades, and (v) cascades triggered by tyrosine kinase receptors. Because these types of signaling pathways are responsible for early stages of development of multicellular animals, their activity in adulthood is limited. Since improper activation of these signaling pathways promotes malignant transformation, development of substances that affect these signaling pathways is an obvious and actively pursued direction for anticancer drug development [17]. This makes the study of intracellular signal transduction pathways that are active in ontogenesis not only important from the standpoint of fundamental biology of development, but also medically significant. *Drosophila* eye development reiteratively uses all these signaling pathways, thus providing researchers with a very promising model system.

THE NOTCH-SIGNALING CASCADE

Notch is a receptor with one transmembrane region, N-terminal extracellular, and C-terminal intracellular domains and initiates the intracellular signaling that is one of the most widely used pathways in multicellular animal development [18, 19] (Fig. 5). Notch regulates cell fate specification during development by increasing the molecular differences between cells. The N-terminal portion of the Notch receptor in *Drosophila* consists of 36 EGF-like repeats and three LNR (lin-12/Notch repeat) repeats [20]. EGF-like repeats number 11 and 12 are responsible for interaction with ligands [21]. The extracellular part of Notch also contains the heterodimerization domain. On the intracellular side Notch contains the RAM-like domain, ankyrin repeats, the transcriptional domain, and the PEST-domain, which regulates the stability of the receptor [22]. In the endoplasmic reticulum (ER) EGF-like repeats of Notch are glycosylated by the *O*-glycosyltransferase Rumi and fucosylated by the *O*-fucosyltransferase, and then *N*-acetylglucosamine is added to the fucose by the Fringe enzyme activity in the Golgi apparatus [23]. The first proteolytic cleavage (S1) of Notch also takes place in the Golgi; this cleavage occurs within the heterodimerization domain

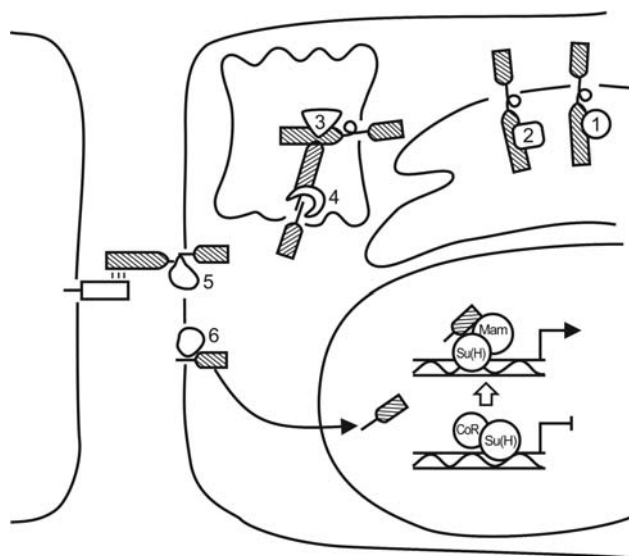


Fig. 5. Notch-dependent signaling cascade: the stages of maturation and intracellular signaling by the Notch-receptor. 1, 2) EGF-like repeats of Notch are glycosylated by the *O*-glycosyltransferase Rumi and fucosylated by the *O*-fucosyltransferase in the endoplasmic reticulum; 3) *N*-acetylglucosamine is added to the fucose by the Fringe enzyme activity in the Golgi apparatus; 4) the first proteolytic cleavage (S1) of Notch at the site located in the heterodimerization region in front of the transmembrane domain is performed by the Furin protease in the Golgi apparatus; 5) binding of a DSL-ligand (transmembrane protein of the neighboring cell) triggers Notch proteolytic S2 cleavage by metalloprotease of the ADAM/TACE/Kuzbanian family that removes the extracellular portion of the receptor; 6) γ -secretase cleaves the intracellular portion of Notch (NICD) which translocates to the nucleus. In the nucleus NICD binds the transcription factor Su(H) and the co-activator Mam, triggering transcription of target genes.

before the transmembrane region and is performed by the protease Furin. This completes the processing reactions, and the ready-to-use receptor, two parts of which are joined by noncovalent bonds within the heterodimerization domain, is delivered to the plasma membrane [24, 25].

Intracellular signaling triggered by the receptor Notch has several key features that distinguish it from other types of signaling cascades. The first such difference is that the ligands for Notch are not secreted molecules, but instead belong to the DSL (Delta, Serrate, Lag2) family of transmembrane proteins. This feature implies that activation of the Notch-dependent signaling occurs through a direct contact of two neighboring cells. Binding of a DSL-ligand triggers proteolytic cleavages of the Notch-receptor, called S2 and S3. The S2-cleavage is performed by metalloproteases of the ADAM/TACE/Kuzbanian family and removes the extracellular portion of the receptor [26]. The subsequent S3-cleavage of the molecule occurs in the lipid membrane layer by the action of γ -secretase — a complex of proteins including pre-

nilin, nicastrin, Aph1, and Pen2, which is also responsible for a similar cleavage of the amyloid precursor protein (APP) and is thus associated with development of Alzheimer's disease [27]. The S3-cleavage releases the cytoplasmic domain of the activated Notch-receptor (NICD). Translocating into the nucleus, NICD interacts with the transcription factor Su(H) [28]. Binding of NICD alters the properties of Su(H), transforming it from a transcriptional suppressor to an activator, directly displacing a co-repressor (deacetylase) and binding the co-activatory proteins of the Mam family. This leads to an increase in transcriptional activity and is associated with recruiting the co-activatory histone acetyltransferase [29]. The best described target genes of this signaling cascade are transcription repressors of the HES/HEY family related to the basic helix-loop-helix family proteins. Furthermore, among the target genes are regulators of cell proliferation Myc and cyclin-D, as well as components of other signaling pathways such as EGFR [29].

The second fundamental difference of the Notch-dependent signaling pathway from most other signaling pathways is the apparent lack of amplification of the signal in its intracellular transmission. Indeed, one molecule of a DSL-ligand binds one molecule of the Notch-receptor, the intracellular portion of which goes after S2/S3-proteolysis into the nucleus and forms a stoichiometric complex with Su(H) and Mam, which stimulates transcription of target genes. However, amplification of the signal is "hidden" in the positive feedback triggered by transcription. The fact is that among the target genes of the Notch-cascade are the Notch gene itself and also genes that stimulate the activity of the Notch pathway [29, 30], whereas transcription of DSL-ligands is indirectly (through downregulation of the transcription factor *achaete/scute*) repressed by Notch signaling [31]. We remind that DSL-ligands are transmembrane proteins, which provide activation of Notch signaling by direct intercellular contacts. If the two contacting cells carry on their surface both DSL-ligands and Notch-receptors, activation of the Notch-cascade is stimulated in both cells (this is achieved through *trans*-activation; it is appropriate to note that *cis*-activation of Notch does not take place; moreover, there exists *cis*-inhibition of the Notch-receptor by DSL-ligands located on the same membrane [32, 33]). However, over time, due to the feedback in the form of stimulated production of Notch and the suppressed synthesis of the ligand, one of the cells, which had initially a slight advantage in the number of Notch-receptors on their surface or their activity, begins to "overplay" the other cell in the level of activation of the cascade. After several rounds of activation of this signaling pathway and the regulated transcription, one of the two initially similar cells is left with a significant level of expression of the Notch-receptor and low expression of DSL-ligands, and ultimately with a significant level of activation of the Notch pathway and expression of its target genes. At the

same time, the Notch-cascade of the second cell is turned off — both because of low level expression of the receptor and other components of the pathway, and because of high level expression of the ligands, leading to *cis*-inhibition of small amounts of the available receptor. This phenomenon is called "lateral inhibition" and is used repeatedly during development of multicellular animals, such as selection of several neuronal precursors from a pool of initially equivalent epithelial cells (see below) [34].

Participation of the Notch-dependent signaling cascade in development of human pathologies. The Notch-dependent signaling pathway is involved in numerous developmental programs both in *Drosophila*, which will be described in the following sections with the example of its eyes, and humans. Mutations in genes encoding components of this signaling cascade are the basis of a number of hereditary or somatic diseases of humans. We will discuss some examples.

T-cell acute lymphoblastic leukemia (T-ALL) is one of the most common forms of leukemia, especially in children. The role of excessive activation of the Notch pathway in development of this cancer was initially detected through analysis of the chromosomal translocation (7;9)(q34;q34.3) in a group of patients with T-ALL. This translocation, as it turned out, leads to formation of a truncated Notch1 gene, expression of which leads to constitutive, ligand-independent activation of the Notch-cascade [35]. Subsequent studies have revealed frequent point mutations in the gene for Notch1, especially in areas that encode its heterodimerization domain and PEST-domain, which controls degradation of NICD [36]. Activating mutations in the Notch1 heterodimerization domain stimulate ligand-independent S2-receptor cleavage, which is accompanied by S3-cleavage and activation of Notch, whereas mutations in the PEST-domain improve the stability of the liberated through the S3-cleavage intracellular Notch (NICD) domain [22, 37]. In general, activating mutations in Notch1 are found in more than half of all cases of T-ALL; in approximately 10–20% of cases simultaneous mutations in the heterodimerization and PEST-domains are found, which speaks for the synergy of these two routes of mutational activation of Notch [22].

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL-syndrome) is a dominant hereditary pathology characterized by recurrent ischemic stroke, migraine, and dementia [38]. CADASIL-syndrome is typically manifested at the age of 45 and by some estimates occurs with a frequency of 1 case per 50,000 people [38]. Genetic studies have revealed that mutations in Notch3 are responsible for the syndrome [39, 40], causing addition or loss of cysteine residues in one of the 34 EGF-repeats of the extracellular domain of Notch3 [41]. It is assumed that it interferes with the normal formation of disulfide bonds in EGF-repeats (normally three bonds per repeat) and the

functioning of the receptor, but it has not been established yet whether these mutations increase or decrease the activity of Notch3 [42]. It is known that in CADASIL-syndrome extracellular parts of Notch3 accumulate on the surface of smooth muscle cells of arterioles, presumably due to multimerization of the mutant form of the receptor due to formation of incorrect intermolecular disulfide bonds [43, 44]. This accumulation causes death of smooth muscle cells of arterioles and as a consequence — loss of cerebral arteries that feed the subcortical neurons, and death of the latter, usually accompanied by fatality in 15-20 years after the onset of symptoms [42].

Alagille syndrome is an autosomal dominant, multi-system pathology characterized by developmental defects of the liver, heart, skeleton, and eyes, which appear in early childhood with a frequency of approximately 1 case per 100,000 infants. Mortality in the Alagille syndrome is 15-20% and in half of the cases occurs as a result of the heart disease (in infants), and in half liver failure due to immaturity of bile ducts (at later stages); there is no treatment for this pathology [45]. In the majority (about 90%) of cases the cause of the syndrome are loss-of-function-mutations in JAG1, encoding a ligand for Notch-receptors [46, 47], and in some cases (6%) — mutations in Notch2 [48]. The dominance of Alagille syndrome is associated with Notch-dependent haploinsufficiency of the signaling cascade: simple reduction in the number of active ligands due to mutations in one of the two alleles of JAG1 (or receptors due to mutations in one of the two alleles of Notch2) leads to a decrease in activity of the Notch-cascade and developmental defects. It is worth mentioning that homozygous mutations in the genes for JAG1, Notch2, and other components of the Notch-cascade are lethal during early embryogenesis.

Insufficient or, conversely, excessive activity of the Notch-dependent signaling pathway underlies many human pathologies. We can mention pancreatic cancer, skin and cervical cancers, spondylocostal dysostosis and hereditary bicuspid aortic valve [42, 49, 50]. These examples underscore the medical importance to study mechanisms of this type of intracellular signaling. *Drosophila* eye provides a very convenient object for such studies, and subsequent sections describe the role of the Notch-cascade in regulation of various stages of development of this organ.

The Notch-pathway in early stages of eye development. Proper eye development in *Drosophila* depends on a clear separation of dorsal and ventral sides of the organ and formation of the equator between the two. These processes are ensured by activation of the Notch-cascade at the boundary between the dorsal and ventral halves of the growing eye. In early imaginal eye-antennal discs, the DSL-ligand Delta is expressed primarily dorsally, and the ligand Serrate — ventrally. In addition, *N*-acetylglucosamine transferase Fringe is also expressed ventrally. Fringe modifies Notch to make it insensitive to activation

by the Serrate-ligand, but stimulating its activation by the ligand Delta. As a result, Notch is activated mainly at the border between the ventral and dorsal compartments, distinguishing this border as a zone of specialized cell—cell interactions and activation of signaling cascades. This is necessary for the growth of the eye, as well as for establishment of the equator as the region of reflection of chirality and orientation of ommatidia [51-53]. Aberrant Notch activity at the border zone leads to defects in the chirality of ommatidia, while complete loss of its activity leads to eye loss [51]. In contrast, excessive activation of Notch produces overgrowth of the eye tissue [51].

Participation of the Notch-cascade in growth of the eye-antennal disc is determined by its role in initiation of morphogenetic furrow (MF) on the posterior pole of the early disc. Activation of Notch at the dorsoventral boundary (which coincides with a posterior pole of the early disc) leads to expression of the transcription factor *Eyg*, which in turn stimulates production of *Unpaired* — a ligand that activates the Jak/STAT signaling cascade and initiation of MF [54].

The Notch-pathway is responsible for specification of R8-cells. In the process of MF progression, activation of the Notch-cascade stimulates transition of cells into the G1 phase and expression of proneural factors *atonal* and *daughterless* [55]. This is accomplished through Notch-dependent suppression of transcription repressors *hairy* and *extra macrochaetae* [56]. Synthesis of proneural factors by a large group of cells within MF marks their commitment to neuronal differentiation, and loss of Notch activity at this stage leads to lack of induction of R8-photoreceptors and neuronal differentiation in general [56, 57]. However, after the passage of MF, *atonal* expression gradually narrows down to clusters and then to individual cells, which become R8 photoreceptors. Loss of expression of *atonal* in many cells of a cluster competent for neuronal differentiation is achieved by the Notch-dependent mechanism of lateral inhibition, and decrease in Notch activity at this stage, in contrast, leads to excessive differentiation of multiple R8-cells [55, 58]. The transition of cells from activating the expression of *atonal* to its suppression in response to stimulation by Notch signaling involves a shift in the mechanism of regulation of expression of this gene. At the stage of MF passage, *atonal* transcription is determined by proteins that bind to its 3'-enhancer, while at the later stages the most defining is the 5'-enhancer which is responsible in particular for the binding of the protein *atonal* itself and thus for self-activation of its transcription [55, 59]. And if the Notch-mediated cascade is able to stimulate expression under the control of the 3'-enhancer, the 5'-enhancer, in contrast, is inhibited by the activity of this signaling pathway [55]. It is interesting to emphasize the importance of self-activation of the proneural gene *atonal*. In other examples of the lateral inhibition, Notch can suppress other proneural genes such as *achaete/scute* [31, 60], but the

ability of such Notch-repressed genes to self-activate appears as a precondition for the functioning of the mechanism of lateral inhibition. The result of Notch activity at this stage of eye development is specification of evenly spaced R8-photoreceptor cells, each of which further recruits their future partners in the ommatidia from the mass of undifferentiated cells.

The Notch-cascade in development of photoreceptor

R7. Differentiation of photoreceptors R1-R6 takes place because of their exposure to EGF secreted by the R8-photoreceptor to build the photoreceptor cluster of nascent ommatidia (see section "The RTK-Signaling Cascade in Photoreceptor Recruitment in Drosophila" of chapter "The Receptor Tyrosine Kinase-Signaling Cascade"). In contrast to these photoreceptors, R7 requires for its specification a double activation: the Ras-dependent signaling cascade and the Notch-cascade. In the absence of the latter, the R7 cell-precursor chooses the R1-R6 fate [12, 61]. Activation of the Notch-cascade in the R7-precursor cells is achieved by expressing the ligand Delta by R1 and R6 cells [12]. Curiously, these cells also express the Notch-receptor, whereas R7 also expresses the Delta-ligand. Moreover, it has been shown that direct activation of Notch signaling in R1-R6 cells turns them into R7 [12, 61]. How do interacting R1, R6, and R7 precursor cells determine which of the two cell fates (R1/R6 or R7) to choose if the choice depends on the Notch-cascade, which can be activated in all three cells? The answer is the lateral inhibition and a specific spatial arrangement of these three interacting cells (see Fig. 3). In an ommatidial precluster the R7-cell is in a physical contact with R1 and R6, while the latter are not in contact with each other. Thus, the R7-cell receives approximately twice more Delta-signal (from R1 and R6) than R1 or R6 (each gets the Delta-signal only from R7). As a result, the Notch-cascade is initially stronger in the R7-precursor, which through lateral inhibition leads to a situation where it is active only in R7 and is not active in R1/R6. As in the cases described above, an important role in Notch-dependent specification of R1/R6 and R7 cells is the *cis*-inhibition by the ligand Delta [62].

The Notch-cascade in development of mechanosensitive eye bristles. Let us consider another mechanism of action of Notch-dependent differentiation that occurs during the development of mechanosensitive eye bristles. Each bristle contains 4-5 cells, which are descendants of a single cell precursor (see above). The precursor cell divides asymmetrically in the anterior-posterior direction. The asymmetry of this division manifests itself in particular in the concentration of protein regulators of Notch signaling Numb and Neuralized on the anterior pole of the dividing cell and in the exclusive inheritance of these proteins by the anterior daughter cells [63-65]. Numb inhibits Notch signaling in the anterior [64], while Neuralized increases the ability of the ligand Delta from the anterior cell to activate Notch in its posterior neigh-

bor [65]. As a result, the Notch-cascade is activated only in the posterior cell, which is responsible for the development of external cell components of the bristle: the hair and the socket. The anterior cell, in turn, produces inner cells (the neuron, the shaft cell and the glial cell; the latter often dies at later stages [66]).

HEDGEHOG-DEPENDENT SIGNAL TRANSDUCTION

Hedgehog (Hh) is a secreted protein first described in *Drosophila*, where Hh was identified as one of the segment polarity genes [67]. Mutations in this gene lead to defects in early embryogenesis with formation of a "lawn" of denticles on the ventral side of the embryo, hence the name of this protein "hedgehog".

Hh is synthesized in the ER as a precursor with a molecular mass of 45 kDa, and completes its processing in the Golgi apparatus. Hh acquires characteristics of a secretory molecule through intramolecular autoproteolysis and lipid modification of the precursor protein. Autoproteolysis is catalyzed by the C-terminal domain of the protein by a mechanism resembling the protein splicing of inteins: the cysteine residue in the conservative Gly/CysPhe string of the proteolysis site plays the role of a nucleophile in hydrolysis of the peptide bond. Cholesterol, serving as an electron donor for this reaction, is recruited by the sterol-binding motif of the C-terminal domain of the precursor protein. The result of this processing is a biologically active Hh-N peptide with the mass of 19 kDa, esterified at the C-terminus with cholesterol [68-70] (Fig. 6).

Biologically active Hh has another lipid modification: palmitoylation on the N-terminus, catalyzed by the acyltransferase Skinny Hedgehog [71]. Such double lipidation is necessary for proper secretion and activity of Hh, but brings about some restrictions both to the process of release of this lipoprotein into the extracellular space and its diffusion between cells. Secretion requires the sterol-sensitive 12-transmembrane protein Dispatched [72]. In the extracellular space monomeric forms of Hh, due to their hydrophobic properties, remain bound to the outer side of the membrane and the extracellular matrix [73]. However, Hh must diffuse over long distances to play the role of the morphogen (a secreted protein, synthesized in a specific zone of a developing tissue and diffusing to form a concentration gradient so that the cellular responses to the received signal depend on the local concentration of the morphogen [74]). It is assumed that this is achieved through special packaging of Hh into particles that mask its lipid parts and have high diffusion capacity. Such particles may be, for example, multimerized Hh aggregates [75] or lipoprotein particles [76]. It is shown that an important role in packaging Hh in such particles is played by the major protein of lipid rafts reg-

gie/flotillin [77]. Segregation of Hh into lipid rafts is observed in Hh-secreting cells and depends on cholesterol modification of this morphogen [78].

Regardless of the molecular form in which Hh is delivered to the signal recipient cell, Hh binds on its surface to the 12-transmembrane protein Patched (Ptc); this interaction is stimulated by co-receptors Ihog and Boi [79–81]. The Ptc receptor constitutively suppresses the activity of another transmembrane protein Smoothed (Smo) [82]. It is assumed that Ptc, as a homolog of several transporter proteins, transports a low molecular weight inhibitor of Smo [83]. Binding of Hh to Ptc inhibits this transport activity, allowing Smo to signal to cytosolic components of this cascade (Fig. 6). Smo is a G protein-coupled receptor (GPCR) by its topology and biochemical activity [82, 84] and is related to receptors of the Frizzled family (see below). However, the role of G-proteins in signal transduction by Smo remains unclear.

The key role in the Hh-dependent signaling cascade is played by a cytosolic complex of proteins organized by the microtubule binding motor protein Costal2 [85, 86] (Fig. 6). This complex also includes the kinases Fused, PKA, CK1, and GSK3 β , as well as the transcription factor Cited1 (Ci) [87]. The function of this cytosolic complex is sequential phosphorylation of Ci,

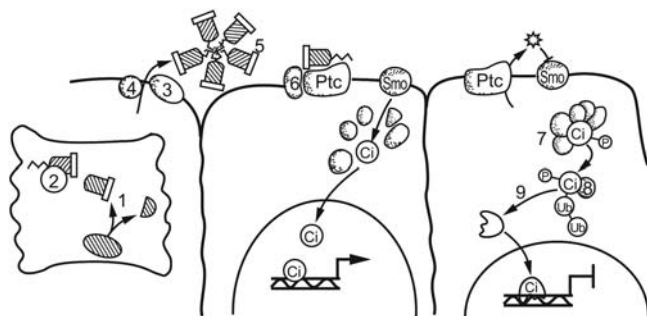


Fig. 6. The Hedgehog-dependent signaling cascade. Hedgehog-synthesizing cell (left): 1, 2) the precursor protein undergoes autoprolysis with addition of a cholesterol residue to the C-terminus and palmitoylation at the N-terminus by the acyltransferase Skinny Hedgehog in the Golgi apparatus; 3) release of the lipidated Hedgehog requires activity of the sterol-sensing transmembrane protein Dispatched; 4, 5) a lipid raft protein reggie/flotillin (4), ensuring packaging of Hedgehog into lipoprotein particles (5), is required for long-range diffusion of Hedgehog. On the receiving cell (center), Hedgehog binds the receptor Patched (Ptc) and co-receptors Ihog and Boi (6), which releases the active GPCR Smoothed (Smo), which in turn provides stabilization of the transcription factor Ci; Ci translocates to the nucleus and triggers transcription of target genes. In the cell, which has not received the Hedgehog signal (right), Ptc acts as a transporter of a low molecular weight inhibitor of Smo. In the absence of Smo activity, a complex of proteins Costal2, Fused, PKA, CK1, GSK3 β forms in the cytoplasm (7) phosphorylating Ci, which leads to its recognition by the ubiquitin ligase Slimb (8) and proteasomal cleavage of the C-terminus of Ci (9). The remaining CiR-fragment enters the nucleus where it plays the role of a transcriptional repressor.

which leads to its recognition by the F-box-containing ubiquitin ligase Slimb. Subsequent ubiquitination of the C-terminus of Ci leads to its cleavage by proteasomes. The remaining fragment of Ci (CiR) is released and enters the nucleus, where it plays the role of a transcription repressor [87, 88]. Active Smo reorganizes the cytosolic complex, preventing phosphorylation and cleavage of Ci. As a result, full-length Ci (CiA) is translocated into the nucleus and serves as an activator of transcription of target genes of the Hh-dependent signaling pathway [87].

Hedgehog-dependent signal transduction and human diseases. As in the case of the Notch-dependent signaling cascade, mutational under-activation of the Hh-signaling pathway underlies a number of genetic diseases – human developmental deficits, whereas mutational hyperactivation of this cascade is responsible for hereditary predisposition to cancer or spontaneous forms of carcinogenesis [89, 90]. For example, a heterozygous mutation in the gene Sonic Hedgehog (Shh, the human Hh homolog) causes holoprosencephaly – malformation of the brain and face with varying degrees of manifestation, from lethal cyclopia to milder facial defects [91, 92]. Holoprosencephaly occurs with a frequency of 4–8 cases per 100,000 births. Interestingly, in rare cases, this defect is caused by a heterozygous mutation in the gene Ptc, which is supposed to be a gain-of-function-mutation and thus also lowers the level of Hh-dependent signal transduction [93]. In addition, approximately 5% of patients with Smith–Lemli–Opitz syndrome (SLOS) also have holoprosencephaly. Other symptoms of SLOS are microcephaly, growth retardation, mental retardation, etc. SLOS is an autosomal recessive disease caused by a mutation in the gene for 7-dehydrocholesterolreductase – the enzyme responsible for a step in cholesterol biosynthesis. It is assumed that this mutation also reduces the effectiveness of the Hh-dependent signaling cascade [89, 90]. It is appropriate to note that hereditary holoprosencephaly and cyclopia are observed in lambs whose mothers ate the leaves of the corn lily (*Veratrum*) during pregnancy [94]. The active substance responsible for these defects was isolated from the corn lily in the 1960s; it was called cyclopamine and found to physically bind Smo and block its activity [95, 96]. Thus, mutational and pharmacological reduction of activity of the Hh-dependent signaling cascade at the later stages of embryo development causes holoprosencephaly.

Greig cephalopolysyndactyly, Pallister–Hall syndrome, type 3 postaxial polydactyly, and VACTERL syndrome can be named among other pathologies caused by mutational decrease of the Hh-dependent signaling pathway activity. All these defects are caused by mutations (hereditary or sporadic) in the genes of Gli3 and Gli2 (*Drosophila* homologs of the transcription factor Ci) [89, 90].

Hereditary or sporadic mutations that increase the activity of the Hh-signaling pathway contribute to car-

cinogenesis. About 40% of patients with Gorlin syndrome have inactivating mutations in the gene *Ptc* (recall that homozygous inactivation of this gene is lethal in early embryogenesis). Since *Ptc* inhibits *Smo* and the whole intracellular cascade, decrease of its amount contributes to ligand-independent activation of this signaling pathway. Patients with Gorlin syndrome have a number of developmental defects of the skeleton, such as polydactyly, overgrowth, fused ribs, and a high frequency of early cancers, such as basal cell carcinoma and medulloblastoma [89, 90, 97]. In addition, most of sporadic cases of medulloblastoma, and virtually all cases of basal cell carcinoma are associated with mutations in the gene *Ptc* [98, 99]. Other forms of cancer, such as cancer of the prostate, breast, pancreas, liver, etc., are also frequently associated with somatic mutations that lead to abnormal activation of Hh-dependent signaling cascade — a decrease in activity of *Ptc*, the overproduction of *Shh*, or mutations in other genes of that signaling pathway [98, 99].

Involvement of the Hedgehog-cascade in early eye development and progression of MF. Hh plays an important role in initiation of morphogenetic furrow (MF) and in its progression [100] (Fig. 2). Hh is synthesized by cells at the posterior pole of the eye-antennal disc, and its diffusion in the anterior direction contributes to the emergence of MF in this point [101]. Furthermore, ectopic expression of Hh causes formation of additional MFs in the early disc [101]. Progression of MF in the anterior direction is provided by the launch of production of Hh by cells differentiating into photoreceptors [102, 103]. One of the Hh target genes in the process of MF progression is another secreted protein Dpp (see below), which also regulates the progression of MF [102]. Another gene whose expression is stimulated by Hh is *atonal*, which triggers neuronal differentiation, the regulation of which by the Notch-cascade is described in the previous section [104]. It is important to note that only undifferentiated cells, located anterior to MF, are competent to respond to the Hh signal. This is achieved through degradation of the key Hh signal transducer, the transcription factor *Ci*, by the action of the Cullin3-containing protein complex in the tissue through which MF has already passed, making it unresponsive to Hh [100, 105]. Thus, reiterative production of the Hh-morphogen is achieved at the peak of MF advancing in the anterior direction.

Dpp- AND TGF β -DEPENDENT SIGNAL TRANSDUCTION

The ligand Decapentaplegic (Dpp) is a member of the TGF β (transforming growth factor β) superfamily of protein signaling molecules and is the *Drosophila* homolog of vertebrate ligands BMP2 (bone morphogenic protein 2) and BMP4. All ligands of the TGF β superfamily act as dimers [106] through receptor serine/threonine

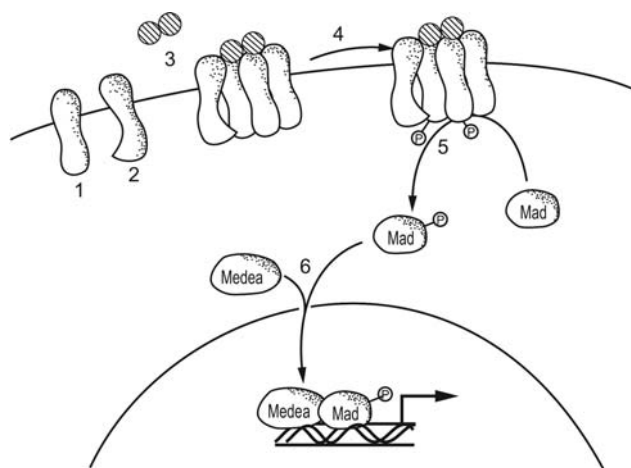


Fig. 7. The Dpp-dependent signaling cascade. Binding of the dimeric Dpp-ligand (3) by the receptor Thick veins (1) and Punt (2) causes their heterotetramerization. The constitutive kinase activity of Punt leads to phosphorylation and activation of Thick veins (4), which is responsible for phosphorylation of the R-Smad protein Mothers against Dpp (Mad, 5). Phosphorylated Mad forms a complex with the co-Smad protein Medea (6) and is transported into the nucleus to trigger transcription of target genes.

kinases with a single transmembrane domain and a cytoplasmic protein kinase domain (Fig. 7). These receptors function as a heterotetramer formed by the binding of the ligand, consisting of two copies of the receptor type I and two copies of the receptor type II [107, 108]. *Drosophila* receptor of the first type for Dpp is Thick veins [109, 110] and of the second type — Punt [111, 112]. Constitutively active receptor type II kinase phosphorylates the receptor type I, activating it. This, in turn, leads to phosphorylation of the cytoplasmic R-Smad-protein: Mothers against Dpp (Mad) in *Drosophila* [113], which makes it competent to bind the co-Smad-protein (Medea in *Drosophila* [114-116]). The heteromeric Smad-complex translocates into the nucleus, where it regulates transcription of target genes.

Links between Dpp/Smad-dependent signaling and human diseases. Mutations in components of the Smad-signaling pathway are the basis of a number of hereditary malformations [117]. For example, the heterozygous genotype for a mutation in the TGF β -receptor 2 (TGFB β R2) is responsible for 10% of cases of Marfan syndrome — a systemic connective tissue disorder, which manifests itself in childhood and is accompanied by disproportional growth, arachnodactyly, lens dislocation, and complications of the cardiovascular system: mitral valve prolapse and aortic dissection [117, 118]. Mutations in TGFB β R1 are also described in Marfan syndrome. In 80% of cases, this hereditary disorder is due to mutations in the gene encoding fibrillin — the main component of the extracellular matrix. It is assumed that the mutation indirectly leads to increased activity of the TGF β /Smad-

signaling cascade. Marfan syndrome clinically overlaps with Loeys–Dietz syndrome, which is also caused by haploinsufficiency in the *TGFBR1* gene. It is curious that in the case of $\text{TGF}\beta$ -receptor mutations in Marfan and Loeys–Dietz syndromes, histological analysis also shows an increase in Smad phosphorylation, suggesting a paradoxical hyperactivation of the $\text{TGF}\beta$ /Smad-signaling cascade in these tissues. Moreover, a partial recovery of the phenotypes can be achieved in a mouse model of Marfan syndrome by artificial lowering of $\text{TGF}\beta$ levels [119]. Thus, congenital defects in Marfan and Loeys–Dietz syndromes seem to be associated with excessive levels of activation of the $\text{TGF}\beta$ /Smad-signaling cascade [117, 118]. Among other hereditary malformations associated with mutations (typically haploinsufficiency) in the gene components of this signaling pathway are hereditary hemorrhagic telangiectasia, thoracic aortic aneurysm type A, Camurati–Engelmann disease, and others [117].

The $\text{TGF}\beta$ /Smad-signaling pathway plays a dual role in the development of cancer [120]. On one hand, this type of intracellular signaling has the tumor-suppressive function in early stages of carcinogenesis, and mutations with reduced activity of this cascade are described in a number of carcinomas [120]. For example, inactivating mutations and deletions in the gene *Smad4* are found in half of all cases of pancreatic cancer [121]. $\text{TGF}\beta$ has even been tested as an adjuvant for anticancer chemotherapy in preclinical models [122]. The tumor-suppressive effect of the $\text{TGF}\beta$ /Smad-signaling pathway is mediated by its ability to limit cell proliferation through the launch of expression of cyclin-dependent kinase inhibitors, such as *INK4B* and *WAF1* [123, 124], as well as by suppression of expression of *c-Myc* [125]. In addition, $\text{TGF}\beta$ is able to stimulate apoptosis by a not yet fully studied mechanism [120, 126].

But on the other hand, $\text{TGF}\beta$ promotes metastasis at later stages of cancer development, for example in cases of breast, colon, and prostate cancer [120, 127]. One of the ways $\text{TGF}\beta$ influences metastasis is its ability to stimulate epithelial–mesenchymal transition [128]. Thus, both reduced and increased activity of the $\text{TGF}\beta$ /Smad-signaling pathway can lead to pathologies in organism development and malignant transformation.

Participation of the Dpp/Smad-signaling pathway in the early eye development. As mentioned above, *dpp* is one of the Hh target genes in the origin and progression of MF [102] (Fig. 2). As in the case of development of *Drosophila* wings [129], Hh, being a poorly diffusing morphogen, induces expression of the highly diffusive morphogen Dpp, which plays a significant role in eye morphogenesis [100]. The Dpp/Smad-signaling pathway suppresses expression of the transcription factor *Homothorax* (*Hth*) anterior to MF [130]. This allows cells to enter into the proneural state and makes them competent to respond to the incoming Hh-signal by the

time MF approaches them. In addition, Dpp inhibits expression of another transcription factor *hairy* in the region directly in front of MF [131], which in turn is necessary for removal of the transcriptional repression of *atonal* – a key regulator of neuronal cell differentiation (see above). The Hh and Dpp morphogens play partially redundant roles in MF progression [102, 131, 132], for example in regulation of expression of the Notch-ligand *Delta* [56].

Wnt/Wingless: THE CANONICAL SIGNALING CASCADE

Wnt-ligands represent a family of secreted lipoglycoproteins, playing key roles in organism development from sponges to humans [133]. The human genome encodes 19 members of this family, and that of *Drosophila* – seven (see <http://www.stanford.edu/~rnutse/wntwindow.html>). The name Wnt reflects the history of discovery of these proteins: the first *Drosophila* **Wnt** (**Wingless**, **Wg**) was cloned as one of the segment polarity genes [134] almost simultaneously with the homologous mammalian gene, identified as the integration site *int-1* of the mouse mammary tumor virus [135]. During embryogenesis, Wnt-proteins function as morphogens: they are synthesized by a specific region of a developing tissue and diffuse forming a concentration gradient which is “read” by the other cells; the cellular responses then depend on the local morphogen concentration [74]. Thus it is not surprising that the processes of secretion and diffusion of Wnt-ligands are tightly regulated [136, 137]. Wnt-synthesizing cells provide a number of posttranslational modifications to the Wnt-protein before it is released into the extracellular space (Fig. 8). Wnts undergo *N*-glycosylation at multiple sites (asparagines 108 and 414 in *Drosophila* Wg) by the action of the ER-localized oligosaccharide transferase complex [138]. The function of *N*-glycosylation may be the regulation of apical secretion of Wnt-proteins by epithelial cells [136, 137]. Biologically active Wnt also has lipid modifications: palmitoylation of the conservative cysteine residue, which lies in the first third of the protein (cysteine 93 in Wg), also occurs in the ER by the *O*-acetyltransferase Porcupine [139, 140]. A mutation in the gene *porc* leads to accumulation of Wnt in the ER and completely prevents the secretion of the morphogen [140, 141]. Additional modification by palmitoleic acid occurs on the conservative serine residue (serine 209 in Wnt3a), but it is unclear whether *Porc* or another acyltransferase is responsible for it [142].

A specialized transmembrane protein *Wntless*/*Evi*/*Sprinter* plays an important role in the process of Wnt-ligand secretion [143–145]. In the absence of this protein, cells of nematodes, fruit flies, and mammals are unable to secrete Wnt-ligands to the extracellular space. *Wntless* delivers Wnt-proteins from the Golgi to the plasma mem-

brane. For the ongoing secretion of new Wnt molecules, maturing in the ER and the Golgi apparatus, Wntless must be returned back to the Golgi. An important role in this recycling of Wntless is played by the so-called retromer complex of proteins; in its absence Wntless accumulates at the plasma membrane and secretion of Wnt is blocked [146-149].

Similarly to the above-described Hh-morphogen, the lipid modifications make Wnt-ligands hydrophobic and in a monomeric form poorly diffusive through the tissue due to their high affinity to the outer side of the membrane and to the extracellular matrix [139, 150]. Thus, both Wnt and Hh face the problem of the long-range delivery, which is necessary for them to function as morphogens. Similarly to Hh, Wnt can package and diffuse in lipoprotein particles to solve this problem [76]. Furthermore, we have identified the special role of reggie-flotillin in the Wnt-producing cells, directing Wnt-secretion into the pathway permitting such packaging [77]. As we show below, the parallels between the Wnt- and Hh-dependent signaling pathways are not limited to the details of secretion and diffusion of these morphogens.

On cell surface, Wnt-ligands bind to two types of coreceptors: 1-transmembrane LRP5/6 (Arrow in *Drosophila*) and 7-transmembrane Frizzled (Fz) (Fig. 8). Fz is related to Smo, which transmits the signal in the Hh-dependent pathway, and like Smo is a G protein-coupled receptor (GPCR) [151-153]. GPCRs represent the largest family of receptors in animals and utilize heterotrimeric G-proteins, consisting of the GDP/GTP-binding $G\alpha$ -subunit and the $G\beta\gamma$ -heterodimer, as the immediate cytoplasmic signal transmitters [154]. In the inactive state $G\alpha$ is associated with GDP and $G\beta\gamma$. Activated GPCR acts as a GEF (guanine nucleotide exchange factor) to catalyze replacement of GDP to GTP. This leads to dissociation of the heterotrimeric complex into $G\alpha$ -GTP and $G\beta\gamma$, each of which is able to activate downstream effector proteins [155]. Fz-receptors primarily bind to and activate heterotrimeric G-proteins of the G_i/o type both in *Drosophila* and in mammalian cells [153, 156, 157]. Another important signal transmitter from Fz is the cytoplasmic protein Dishevelled (Dsh) [158]. Dsh binds to the C-terminus of Fz [159], while the heterotrimeric G-proteins usually interact with the intracellular loops of GPCR [160]. It remains unclear whether the simultaneous interaction of Fz-receptor with both types of intracellular transmitters is possible. It should be noted however that in a number of genetic experiments Dsh acts downstream from heterotrimeric G-proteins in Fz signal transduction [156, 161]. A possible explanation for this phenomenon has been provided by studies demonstrating the physical interaction of Dsh with $G\beta\gamma$ [162-164]. $G\beta\gamma$, released along with the $G\alpha$ -subunit of the initially trimeric G-protein by the GEF activity of Fz-receptors, remains associated with the plasma membrane

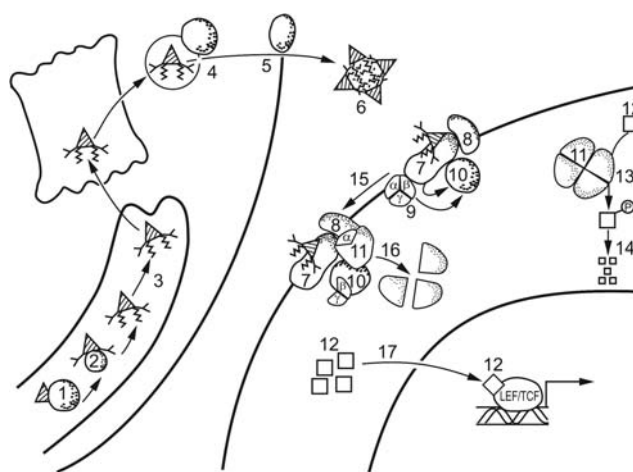


Fig. 8. Wnt/Dishevelled: the canonical signaling cascade. In the Wnt-synthesizing cells (left), the Wnt-ligand undergoes several post-translational modifications in the endoplasmic reticulum: *N*-glycosylation by the oligosaccharyltransferase complex (1), palmitoylation by the *O*-acyltransferase Porcupine (2), and additional modification by palmitoleic acid (3). The Wntless protein plays a key role in transportation of Wnt from Golgi to the plasma membrane (4). Wnt-ligands can be packed into lipoprotein particles (6); an important role in this process is played by the protein component of lipid rafts reggie-flotillin (5). In the Wnt-responding cell (right), Wnt binds to the GPCR Frizzled (7) and 1-transmembrane receptor LRP5/6 (8). The key intracellular signal transmitters are heterotrimeric G-proteins (9) and Dishevelled (10). In the absence of their activity, Axin (11) organizes a complex of proteins, which binds β -catenin (12) leading to its phosphorylation (13) and proteasomal degradation (14). Under the influence of $G\alpha$ -subunits of heterotrimeric G-proteins, Dishevelled and LRP5/6, Axin is relocated to the membrane (15) and displaced from the destruction complex (16). As a result β -catenin accumulates and translocates into the nucleus (17) to activate transcription in coordination with LEF/TCF.

through its lipid modifications [165] and recruits Dsh from the cytoplasm, allowing it to interact with Fz [163].

The intermediate outcome of action of these intracellular signal transmitters is restructuring of the so-called destruction protein complex, which includes Axin, APC, casein kinase (CK), and glycogen synthase kinase 3 β (GSK3 β) [166]. The function of this complex is binding and sequential phosphorylation of cytoplasmic β -catenin, which leads to its ubiquitination and subsequent proteasomal degradation [167] (Fig. 8). It is appropriate to note another similarity with the Hh-dependent signaling pathway, up to the involvement of the same kinase (GSK3 β) both in the Hh-dependent complex, cleaving Ci, and in the Wnt-dependent complex, degrading β -catenin.

The key role in reorganization of the destruction complex triggered by Wnt is played by interaction of Axin, organizer of this complex, with the receptor LRP5/6 and intracellular signal transmitters Dsh and the $G\alpha$ -subunit of the heterotrimeric Go protein. These interactions displace other components of the complex. Axin is a protein

with multiple defined domains and a number of disordered regions, providing a platform for binding multiple partners [168, 169]. So, G α is able to physically interact with the RGS-domain of Axin [163], probably inhibiting the binding of APC, which is a component of the destruction complex [170]. On the other hand, Dsh binds to the DIX-domain located on the opposite side of Axin [171]. Thanks to the combined action of Dsh and G α , Axin is transferred to the membrane [163, 172], where it finally starts to interact with the cytoplasmic tail of LRP5/6 [173]. This interaction is stimulated by phosphorylation of LRP5/6 by CK and GSK3 β [174, 175]. As a result of these coordinated actions, Axin-dependent destruction complex is rearranged and becomes unable to bind and phosphorylate β -catenin. As a result, the latter gradually accumulates in the cytoplasm and diffuses into the nucleus, where it interacts with a number of transcriptional cofactors, first of all LEF/TCF, to induce transcription of target genes of the Wnt-dependent signaling pathway [176].

Endocytosis plays an important role in the Wnt-cascade. Unlike the majority of GPCRs, internalization of which leads to termination of intracellular signaling, internalization of Fz-receptor serves to amplify the signal [177]. An important role in the process of signal amplification is played by the small GTPase Rab5, the central regulator of early stages of endocytosis [177, 178]. This G-protein is recruited by the G α -subunit of the heterotrimeric protein Go from the cytoplasm to the plasma membrane, where its GEF-regulators are localized [178]. Once in proximity to Fz-receptors, Rab5 stimulates their internalization into early endosomes. Through an as yet not fully understood mechanism, in this compartment the Fz-receptor (or rather the Wnt-Fz-LRP5/6 complex) produces a greater impact on reorganization of the Axin-dependent destruction complex. It is possible that different components of Wnt-dependent signal transduction are involved differently in transmission of the signal from the plasma membrane versus the early endosomes [153, 178]. This mechanism can be used by cells for differential responses to different concentrations of the Wnt-ligand, or to different ways of its packaging and presentation [153].

The Wnt-dependent signaling pathway and human pathology. Because the Wnt-dependent signaling pathway plays an important role in organism development, it is not surprising that mutations in genes encoding components of this cascade are the basis for a number of hereditary diseases and developmental malformations [179]. For example, mutations in LRP5 are responsible for defects in development of skeletal mass in humans: activating mutations lead to excessive skeletal mass and inactivating – to a decreased bone mass and osteoporosis [180–182]. Dominant mutations in the gene Fz4 are responsible for development of familial exudative vitreoretinopathy – a vascular disease of the retina, accompanied by peripheral vitreous opacities and retinal exudates in the

retina [183]. The reason for the dominance of this mutation is production of a truncated Fz4, which oligomerizes with the normal receptor and retains it in the ER [184]. There are a number of other hereditary diseases associated with malfunctioning of the Wnt-cascade [179].

In the adult organism Wnt-cascade is mostly off. However, both under-activation and excessive activation of this signaling pathway, e.g. due to somatic mutations, lead to pathologies. Excessive activation of the Wnt-pathway in a number of tissues contributes to carcinogenesis [185]. About 50% of all breast cancer cases are associated with aberrant activation of the Wnt-cascade, for example due to overproduction of Wnt-ligands or loss of production of natural Wnt-antagonists such as Dickkopf and sFRP (secreted frizzled-related protein) [186]. Mutations in the genes APC and Axin are the basis for colon cancer: more than 90% of all cases of this disease are associated with such mutations [187]. Other tissues are also not insured from carcinogenesis resulting from somatic hyperactivation of this cascade: mutations in its components are described in many cases of stomach, ovarian, prostate, and other cancers [187].

On the other hand, the proliferative function of the Wnt-dependent signaling pathway defines the role of activation of this cascade in regeneration of various tissues after injury, and lack of activation of this pathway is supposed to prevent the full restoration of mammalian tissues [188]. Therapeutic approaches to stimulate regeneration are based on direct stimulation of this cascade, for example in case of bone repair after fractures [189]. However, excessive Wnt-dependent proliferation can also cause pathologies, distinct from carcinogenesis described above. For example, excessive Wnt-dependent proliferation of vascular smooth muscle cells can lead to thickening of arterial walls and clogging of blood vessels; increased activity of this cascade is also responsible for cardiac hypertrophy [190].

Finally, the Wnt-signaling pathway is important in neuronal remodeling [191], and deficiency in the Wnt-cascade activation in neurons is associated with development of neurodegenerative diseases such as Alzheimer's disease [192]. It has been shown that the β -amyloid peptide is able to physically bind Fz receptors and prevent activation of Wnt-dependent signal transduction [193]. In addition, it has been shown that directed activation of the Wnt pathway has neuroprotective effects in cultured neuronal cells treated with β -amyloids [194, 195].

Thus, the right balance between insufficient and excessive levels of activity of the Wnt-dependent signaling pathway is important for normal functioning of tissues [196]. Studies of molecular mechanisms of this cascade can identify new components that can serve as targets for directed activation or deactivation by low molecular weight drugs [197]. The Wnt-cascade plays an important role in eye development in *Drosophila*, which is an ideal object to study this signaling mechanism.

Wnt/Wingless-dependent signal transduction in early development of *Drosophila* eyes. Wg performs several functions during development of the eye-antennal disc, such as the supply of positional information for the disc morphogenesis, stimulation of differentiation of head tissues, etc. [198]. In the first larval instar, Wg acts in the dorsal part of the eye-antennal disc [199] promoting expression of homeodomain genes of the Iroquois complex, in particular mirror [200]. These genes suppress dorsal expression of Fringe, the glycosyltransferase of the Notch-receptor [51–53]. As described in section “The Notch-Pathway in Early Stages of Eye Development” of chapter “The Notch-Signaling Cascade”, exclusive expression of Fringe in the ventral part of the disc plays an important role in specification of the equator and the choice of the point of initiation of the morphogenetic furrow (MF).

In the second larval instar, Wg is present throughout the disc, whereas in the third larval instar the zone of Wg expression is limited to lateral margins of the eye part of the disc [201, 202]. Wg-dependent signal transduction contributes to formation of the head capsule at the expense of the eye cells: directed activation of the Wg cascade leads to transformation of the eye tissue into the head cuticle, whereas mutational suppression of this signaling pathway leads to an increase in the eye size [203, 204]. The gradient of Wg, decreasing towards the equator, prevents incorrect initiation of MF (Fig. 2) [205, 206]. The Wg-cascade control over cell differentiation into the head capsule at the expense of the eye tissue is determined by regulation of MF formation: lowering Wg activity transforms head capsule into the ectopic eye tissue due to initiation of ectopic MF. In contrast, mutational activation of the Wg-cascade inhibits initiation of the normal MF, blocking differentiation of the eye; eye disc cells form excessive cuticular tissue of the head in this case [203–206].

The role of the Wingless-cascade in formation of peripheral structures of the eye. Normal ommatidia of *Drosophila* eyes are separated from the cuticle of the head capsule by a number of specific structures. On the border with the head capsule, the eye is surrounded by the “pigment rim” (PR) – a narrow stripe of tissue consisting entirely of pigmented cells that insulate the eye from side-wise illumination [9]. In addition, the so-called “dorsal rim” (DR) lies on the dorsal side behind PR. DR is a row of ommatidia specialized for polarized light detection due to the particularly large central rhabdomeres of the R7 and R8 cells, both of which express the UV-sensitive opsin-3 (see introductory section “Structure and Function of the *Drosophila* Eye”); DR ommatidia also have unusual neuronal projections to the optic ganglion [9, 207]. Wg secreted by the head capsule plays the key role in formation of both PR and DR [9, 208]. PR is formed at the pupal stage as a result of apoptosis of non-pigment cells of ommatidia aligning the head capsule

[209]. In general, Wg can induce apoptosis of differentiated eye cells [9, 209–211], and one of the earliest identified alleles of *wg*, *glazed*, leads to eye reduction through death of non-pigment cells induced by ectopic Wg expression [212]. Additionally, ectopic Wg causes the “mirror” eye phenotype due to loss of the antireflective nanostructures of the corneal surface [2]. Formation of DR requires synthesis of the transcription factor Hth induced by Wg diffusing from the head capsule; lower concentrations of Wg are required for DR as compared to PR induction [9, 208].

Wingless diffusion sets the gradient of the “factor X”.

In addition to the described above functions of Wg in early dorsal–ventral specification of the eye disc (see the section “Wnt/Wingless-Dependent Signal Transduction in Early Development of *Drosophila* Eyes”), Wg plays an important later role in formation of the equator and chirality of ommatidia. Diffusing deep into the eyes from dorsal and ventral sides of the head capsule, Wg forms a downward concentration gradient with a minimum at the equator. Formation of a clone of cells ectopically synthesizing Wg can re-polarize ommatidia in a non-autonomous way: ommatidia located on the polar side (towards the head capsule) from Wg-producing cells change their chirality and orientation in a mirror-reflection and form an ectopic equator at the boundary with the normally-oriented ommatidia [213, 214]. The effectiveness of such non-autonomous repolarization is the stronger, the farther away from the head capsule the clone localizes. Similarly, formation of a clone of cells that are unable to transmit the signal from Wg, for example due to loss of Wg-components of the cascade such as Arrow, Dsh, or β -catenin, re-polarizes ommatidia – but on the equatorial side of the clone, the stronger the closer to the poles (head capsule) clones localize [214]. On the basis of these data a hypothesis has been formulated that the concentration gradient of Wg from the pole to the equator induces the counter gradient of “factor X” – secreted or transmembrane substance(s), which determines activation of Fz-dependent “non-canonical” signaling cascade that determines planar polarity of ommatidia [214, 215]. This signaling cascade is discussed in the next section.

THE NON-CANONICAL Frizzled/PCP-SIGNALING CASCADE

In addition to the canonical, β -catenin-dependent signaling cascade, which regulates transcription, Fz-receptors are capable of activating non-canonical signaling cascades [216]. The best studied among them is the cascade that regulates planar cell polarity (PCP). The Fz/PCP-signaling cascade directs “horizontal” polarization of epithelial cells in the plane of the tissue, perpendicular to the “vertical” apical-basolateral polarization of epithelia. This depends on the proper polarization of the

cytoskeleton and is independent from changes in the transcriptional profile of cells [217]. PCP is manifested morphologically, for example, by formation of actin-rich hairs by *Drosophila* wing cells; each hair grows in the distal direction. In mammals, PCP manifests itself, for example, in polarization of hair cell stereocilia in the Corti organ of the inner ear. Cells participating in PCP respond by polarization of their cytoskeleton to the extracellular polarizing information. In this sense, PCP is similar to chemotaxis of leukocytes and directed growth of yeast cells stimulated by a pheromone [218, 219]. In all these events, polarization of the cytoskeleton is triggered by GPCRs, in the case of PCP – Fz [220].

However, the question of which ligand activates the Fz/PCP-signaling cascade remains open. While in vertebrates involvement of “non-canonical” Wnt-ligands such as Wnt5a, Wnt11, and others in activation of PCP has been demonstrated [221, 222], experiments in *Drosophila* have revealed that neither Wg nor other Wnt-ligands represent the “factor X” – the mysterious activator of the Fz/PCP-signaling pathway [223, 224]. Studies of the role of proteins Fat, Dachshous, and Four-jointed in the regulation of PCP were launched some years ago. These proteins form gradients of expression and activity in developing tissues and thus are well-suited for the role of “factor X” [225, 226]. However, genetic experiments in *Drosophila* clearly demonstrated that Fz and Fat/Dachshous organize independent signaling pathways redundantly coordinating PCP [227].

How is the intracellular signal transduction in the Fz/PCP-signaling cascade organized? Fz-receptors, initially uniformly distributed in the apical plasma membrane, accumulate with the development of PCP on one pole (distal in the case of *Drosophila* wing cells), determining the future place of activation of the actin cytoskeleton (and hair outgrowth in the wing cells) [228]. Apparently, proteins involved in PCP can be divided into Fz-signal transmitters and signal amplifiers; mathematical modeling of PCP also separates the Fz/PCP-signaling cascade into two phases: receiving the transient polarizing signal, and subsequent establishment of cell polarization on the basis of massive re-localization of protein components of PCP [229]. Direct transmitters of the Fz/PCP-signal, as in the canonical Wnt-dependent pathway, are apparently heterotrimeric G-proteins and Dsh [156, 230]. Proteins that play an important role in the subsequent re-localization of Fz-receptors are Prickle, Van Gogh, Diego, and a 7-transmembrane protein Flamingo [231–235]. In addition, re-localization of Fz depends on small Rab-proteins that regulate vesicular transport, namely Rab5 described above (see chapter “Wnt/Wingless: the Canonical Signaling Cascade”) and Rab11 regulating slow recycling of endosomes to the plasma membrane [178]. Apparently, Rab5-dependent endocytosis is required for microtubule anchoring and transport of Fz-containing endosomes in the distal direction

[178, 236, 237], while Rab11-dependent recycling is required for the release of Fz at the distal membrane [178]. Finally, small G-proteins of the Rho family are responsible for execution of the cell polarization response program: actin growth of hairs on the wing of *Drosophila* and rotation of ommatidia in the eye [238].

The Frizzled/PCP-signaling cascade and human pathology. In vertebrates, PCP is involved in neural tube closure [239]. Mutations in PCP-components in animal models cause defects in this process of late embryogenesis [217, 239]. In humans, mild defects in neural tube closure occur in 1–2 infants per 1000, and in some cases are associated with mutations in the gene *Vangl1* (homolog of *Drosophila* Van Gogh) [240]. The Fz/PCP-signaling cascade is also involved in carcinogenesis, but, in contrast to the canonical β -catenin-dependent cascade that can cause malignant transformation, Fz/PCP is involved in later stages of cancer progression, such as invasiveness and metastasis of cancer cells [241]. For example, Wnt5a increases aggressiveness of metastatic gastric cancer and melanoma, directly stimulating migration of cancer cells [242, 243] – yet another parallel between the PCP and chemotaxis [218].

The Frizzled/PCP-signaling cascade in regulation of ommatidial polarity in *Drosophila*. A *Drosophila* ommatidium in a cross-section forms an asymmetrical trapezoid, where the photoreceptor rhabdomere R3 stands aside from the rest of the rhabdomeres (Figs. 1 and 4). Thus, ommatidia have chirality, which is the same for all ommatidia in the dorsal half of the eye and is mirrored in the ventral ommatidia. In addition, all ommatidia are oriented with their R3-tops towards the poles of the eye (Fig. 4). Chirality and orientation of ommatidia is determined by the level of activation of the Fz/PCP-signaling pathway in progenitor cells of photoreceptors R3 and R4. In an ommatidial precluster, these cells initially occupy symmetric positions on opposite sides of the precluster, so that one of them is located closer to the equator, and the other – closer to the pole of the eye [215]. As the concentration of “factor X” is supposed to be at maximum at the equator and to decrease towards the poles, the progenitor cell initially closest to the equator receives more “factor X” signal [215]. At subsequent stages of precluster development, R3/R4 precursor cells engage in a physical contact with each other and “compare” their levels of activation of the Fz/PCP-cascade. This comparison is achieved by the negative impact of Dsh on Notch [244]: Dsh, activated by the Fz signaling pathway, physically binds to the cytoplasmic domain of Notch and directs this receptor to a degradation compartment [245]. As in *Drosophila* wings, the Fz/PCP-signaling cascade in R3/R4 cells leads to re-localization of components of the cascade, so that the future R3-cells accumulate Fz and Dsh at the border with the future R4, while the latter locates them on the opposite side, beyond the contact zone with the neighbor cell [246, 247]. This also helps to

selectively reduce the number of Notch-receptors in the future R3 on its border with the R4. And next the much discussed above process of lateral inhibition (see chapter “The Notch-Signaling Cascade”) is engaged again to amplify the differences in activation of the Notch-cascade between neighboring cells [215, 248, 249]. As a result, the cell that had a greater level of activation of the Fz/PCP-pathway completely inhibits the Notch-cascade, becomes the R3-photoreceptor, and pulls itself away from the tight ommatidial precluster. The second cell becomes the R4.

THE RECEPTOR TYROSINE KINASE-SIGNALING CASCADE

The human genome encodes 58 transmembrane receptors with tyrosine kinase activity, distributed over 20 functional groups [250], including the family of epidermal growth factor receptors (EGFR), the insulin receptor family, the family of fibroblast growth factor receptors (FGFR), etc. In most cases, receptor tyrosine kinases (RTK) are monomers dimerizing upon binding of the ligand. The *Drosophila* genome encodes only 15 RTKs. However, studies on this model organism have played a key role in the deciphering of the RTK-signaling cascade. Therefore, we deviate from the standard structure of our review and describe this cascade on the example of development of the R7-photoreceptor in *Drosophila* and the role of intracellular signaling initiated by the RTK Sevenless in this process (Fig. 9).

The first human RTK, EGFR, was cloned in 1984. Its amplification in cells of epidermoid carcinoma and its homology to the *v-erb-B* oncogene of avian erythroblastosis virus immediately pointed to the potential role of the RTK in carcinogenesis (see below) [251]. Sevenless (Sev), the first *Drosophila* RTK, was cloned in 1987 [252]. Initially, a mutation in the *sev* gene was obtained in the laboratory of Seymour Benzer in a search for mutants defective in phototaxis [253]: wild-type flies prefer UV light in a T-maze, while *sev* mutants lose this preference. Subsequent work showed that the photoreceptor R7 bearing the UV-sensitive opsin was absent in the mutant [254] and was replaced by an additional cone cell [255]. The ligand for Sev turned out to be the transmembrane protein Bride of Sevenless (Boss), expressed by the R8-photoreceptor to activate the neighboring R7-cell [256]. Further experiments to search for mutations that could rescue the *sev* phenotype laid the architecture of the RTK-signaling cascade. Thus, the kinase activity of Sev and its ability to autophosphorylate has been shown necessary for the formation of R7-photoreceptors [257, 258]. Son of Sevenless (Sos), which was identified as the guanine nucleotide exchange factor for the small GTPase Ras1 [259-261], emerged as the key intracellular transmitter of the signal from Sev. Moreover, the activated form of Ras1 was able to

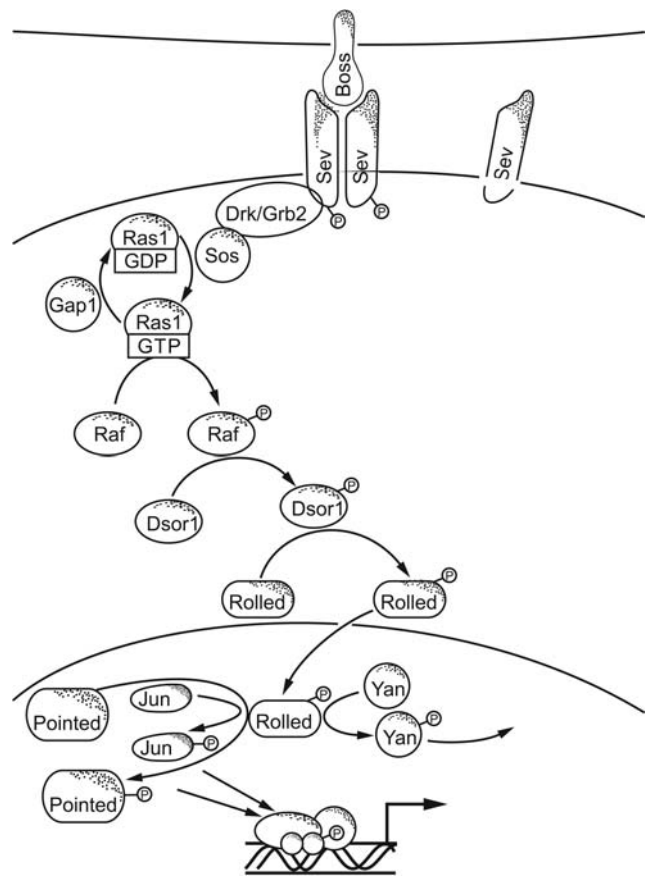


Fig. 9. The receptor tyrosine kinase-signaling cascade with Sevenless as an example. Binding of the transmembrane ligand Bride of Sevenless (Boss) to the receptor tyrosine kinase Sevenless (Sev) leads to dimerization and autophosphorylation of the latter. Phosphorylated Sev is recognized by the adapter protein Drk/Grb2, which recruits the guanine nucleotide exchange factor Son of Sevenless (Sos) from the cytoplasm. Sos activates the small G-protein Ras1 (the opposite action is played by Gap1). GTP-bound Ras1 activates the MAP kinase signaling cassette, consisting of the kinases Raf, Dsor1, and Rolled, successively phosphorylating and activating each other. The active form of the MAP kinase Rolled is translocated into the nucleus where it phosphorylates transcriptional activators Pointed and Jun, as well as the transcriptional suppressor Yan.

rescue the loss of R7 in *sev* and *boss* mutants and also induced formation of multiple R7-cells [262].

The SH2/SH3 domain-containing adapter protein Downstream of Receptor Kinase (Drk/Grb2) plays a central role in the activation of Sos and Ras1 by the active (i.e. autophosphorylated) form of Sev. Drk/Grb2 binds both to Sos and phosphorylated Sev (the latter through its SH2-domain) bridging the two proteins [263, 264]. While guanine nucleotide exchange factors activate G-proteins, GTPase activating proteins (GAP) perform the opposite function accelerating the transition of G-proteins into the inactive GDP-bound state. Gap1 was isolated as a negative regulator of Ras1 and the Sev-dependent signaling cascade [265]. Finally, Raf, the first kinase of the

MAP (mitogen-activated protein)-kinase complex, was identified as an essential component of the RTK-cascade, activated by Ras1 and capable upon constitutive activation to promote the formation of R7-cells even in the absence of Sev [266]. The subsequent kinases of the MAP-kinase complex – MAPKK Dsor1 (Downstream of raf1) [267] and MAPK Rolled [268] – are also necessary for the specification of R7. Among targets of the MAP-kinase Rolled are nuclear transcription activator proteins Jun [269] and Pointed [270], as well as the repressor Yan [270]. Phosphorylation by the MAP-kinase triggers transcription of several target genes, including the gene *phyllopod* [205], which together with another nuclear protein Sina is needed for further steps of specification of the R7-photoreceptor [262, 271]. A number of additional regulators of the RTK-signaling pathway have been identified in addition to the components described here [272].

Participation of RTK signaling pathways in human diseases. Cloning of the first RTK (EGFR) revealed a potentially important role of this type of signal transmission in carcinogenesis [251]. A vast array of subsequent studies has confirmed this role. There are two principal ways of cancerous transformation due to hyperactivation of RTK in human cells. The first is excessive production of RTK by gene amplification, the second – activating mutations in the RTK genes [273]. For example, the genes of receptors EGFR and ErbB2 are often amplified in lung carcinoma and breast cancer [273, 274]. In particular, 30% of the breast cancer cases amplify the ErbB2 gene 2-20 times, and the level of ErbB2 overexpression correlates with the aggressiveness of the tumor and reduced patient survival [275]. Activating mutations in EGFR have been described in human glioblastoma cells [276]. Activating mutations in the RTK Ret underlie hereditary cancer syndromes: multiple endocrine neoplasia 2A and 2B, and familial medullary thyroid carcinoma [273]. Many other cases of amplification and activating mutations in the RTK genes, contributing to carcinogenesis in different tissues, have been described [273, 274].

The active center of kinases is very convenient for development of small molecule inhibitors, which are typically based on ATP analogs (such convenience of target proteins is called “druggable”). Thus pharmaceutical companies are actively searching for anticancer drugs that block the activity of various RTK. A significant number of RTK inhibiting anticancer drugs are already on the market [273, 274, 277]. Here are some examples:

- Imatinib (Glivec) from Novartis inhibits among other targets the RTK c-KIT and is used in particular for the treatment of c-KIT-positive stromal tumors of the gastrointestinal tract [278];

- Iressa (Gefitinib) from AstraZeneca inhibits EGFR and is used to treat non-small cell lung cancer [279];

- Pazopanib (Votrient) from GlaxoSmithKline acts among other targets on the RTK VEGFR (vascular

endothelial growth factor receptor) and is used to treat advanced renal cell carcinoma [280];

- Lapatinib from GlaxoSmithKline acts on EGFR and Erb2 and is used to treat Erb2-overexpressing metastatic breast cancer [281].

The RTK-signaling cascade in *Drosophila* photoreceptor recruitment. We have described above the role of signaling by the RTK Sev in formation of photoreceptor R7. In the absence of this signal the R7-precursor does not choose the neural fate and instead becomes a cone cell [255]. However, RTK-dependent signal transduction plays a more general role in neuronal differentiation in the developing eye. Specifically, the RTK EGFR and its secreted ligand Spitz are needed for sequential recruitment of photoreceptors R1-R6 and then the remaining ommatidial cells into the ommatidial precluster (see Fig. 3) [100]. Removal of EGFR or Spitz leads to formation of dramatically reduced eyes containing only R8-photoreceptors, which are first to differentiate and are the only cells not requiring activation of the RTK-cascade for their appearance [282, 283].

R8-cells are the first to start synthesizing Spitz, which stimulates neighboring cells for neuronal differentiation; these cells become photoreceptors R2 and R5. The latter also begin to produce Spitz, which promotes recruitment of R3 and R4, and then R1 and R6 (Fig. 3). Artificial activation of EGFR can even compensate for the absence of Sev in the R7 cell precursor [282].

Not only photoreceptors but also cone cells require activation of the Spitz-EGFR cascade for their differentiation [282]. Why, then, do cone cells not become photoreceptors of the R1/6 type? Because, unlike the R1/6 cells, cone cells also activate the Notch-cascade through contact with Delta-expressing photoreceptors (Fig. 3) [12]. But then why do cone cells not become R7-photoreceptors, which, as described above, require both the Notch- and RTK-signal for their specification (the latter through the Boss-Sev interaction)? Because, in contrast to the R7-cells that receive high levels of the RTK activation cascade, activation of this cascade in cone cells achieved through the interaction of Spitz with EGFR is much weaker [12].

In addition, an important role in cell differentiation in the developing eye is played by Argos, an extracellular inhibitor of EGFR [284], secreted by cells in response to activation of the EGFR-signaling cascade [285] and having a higher diffusion capacity in comparison to Spitz [286]. Based on these data a hypothesis has been formulated on the synthesis of a poorly diffusible activator and a well-diffusing EGFR inhibitor, achieved in expanding concentric waves around R8, capturing all the new cells in a growing ommatidial cluster [282]. This model also predicts the importance of the temporary component in RTK-stimulated differentiation: cells recruited earlier become photoreceptors, the later ones become cone cells, and even later – pigment cells [282].

In addition to regulating differentiation, activation of the RTK-signaling cascade through the Spitz–EGFR ligand–receptor pair is important for preventing apoptosis in the *Drosophila* eye cells, including secondary and tertiary pigment cells [283, 287].

METHODS FOR STUDYING EYE DEVELOPMENT IN DROSOPHILA

Numerous data described in previous sections were based on well-developed histological, immunochemical, and microscopic techniques of analysis of both eye-antennal larval discs and cross-sections of adult fly eyes, polished by many laboratories. However, not these necessary methodologies make the fruit fly such a convenient object for studies, but the unique technologies of genetic manipulation. Over a hundred years of radiation, chemical, and P-element mutagenesis in combination with the compactness of the *Drosophila* genome have created a huge collection of mutant lines that cover most of the genes of this insect. The very development of these methods has given a tremendous impetus to further investigations and led to breakthrough discoveries of general biological significance (see, e.g. [67]).

Many (and often the most interesting) mutations are homozygous lethal; the lethality sometimes occurs too early in embryogenesis to study the role of the corresponding genes in eye development. To circumvent this problem, researchers have developed the FLP/FRT-system to produce somatic clones, homozygous mutant for the desired gene [288, 289]. FRT (FLP recombinase recognition target)-sequences have been integrated in various positions of *Drosophila* chromosomes. The investigated mutant allele (e.g. an allele of the gene *arrow*, located at the cytological position 50A9-50A11 of the right arm of the second chromosome) is recombined with the FRT-sequence located proximally on the chromosome (for example, FRT42D, located at the cytological position 42D of the same chromosome). Next, a marker gene (in eye experiments it is often the gene *white*, encoding the transporter of pigment for pigment cells and photoreceptors) is integrated distally from the FRT (with the endogenous copy of the *white* gene of the first chromosome being mutated). Finally, the key gene in this experimental approach, the yeast flippase (FLP), is integrated into the genome of *Drosophila*, for example, under the control of the heat shock protein promoter (hs-FLP). To generate somatic clones, young larvae (e.g. of the age of 24-48 h after egg laying) heterozygous for the mutation under study are collected. In our example involving the *arrow* gene, they would be of the following genotype: *white*[−], *hs-FLP*; *FRT42D*, *arrow*²/*FRT42D*, *white*⁺. Heat shock (1 h at 37°C) causes expression of flippase, which stimulates site-specific recombination between the two FRT-sites in dividing somatic cells. As a result the

heterozygous parent cell produces one daughter cell homozygous mutant for *arrow*², and one homozygous wild-type for *arrow*. Simultaneously, the first cell loses the marker *white*⁺. Dividing, these cells form clones. The clones identified in the whole eye and on histological cross-sections by the absence of pigment will be completely devoid of the Arrow protein (the co-receptor for Wg), which allows studying its role and the function of the Wnt-dependent signaling cascade in general in eye development [9, 214]. It should be added that embryos homozygous for mutant *arrow*² are defective in establishment of segment polarity and die without even producing first instar larvae [290]. In addition to hs-FLP inducing expression of flippase everywhere under the influence of a heat shock, one can use more tissue-specific constructs such as Ey-FLP expressing flippase under the control of regulatory sequences of the gene *eyeless* [291].

A huge role in the development of *Drosophila* genetics was played by the technology of transgenesis – incorporation of foreign genes into the genome of the insect. This method uses the enzyme and sequences of transposable elements (P-elements) for the random integration of the desired DNA into the genome [292]. Recently new methods for directed integration into defined loci have also been developed [293-296].

Another technology that has tremendous value for *Drosophila* genetics was also established on the basis of the introduction of foreign yeast sequences into the insect genome. It is the famous Gal4/UAS-expression system [297]. The desired gene (*Drosophila* or human, wild-type or mutant) is cloned into a cassette containing the UAS sequence (upstream activation sequence: an enhancer element not recognizable by any *Drosophila* transcription factors), and this cassette is integrated into the genome. Crossing this UAS-X line with a line expressing the yeast transcription factor Gal4 results in expression of the gene X. Having a large collection of various Gal4-lines, researchers can achieve expression of the desired transgene in virtually any desired tissue and at any desired stage of development. Here is a list of lines used in the studies of eye development (for a more complete list see <http://flystocks.bio.indiana.edu/Browse/misc-browse/gal4.htm>; <http://flybase.org/>):

- GMR-Gal4: the construct contains five repetitive elements of the gene promoter *Rh1*, interacting with the transcription factor Glass; it causes expression in postmitotic cells of the eye [282];
- Ey-Gal4: contains an enhancer gene *eyeless*, expression occurs in the early eye cells before MF [298];
- Sev-Gal4: contains the enhancer of the gene *Sevenless*; expression is observed in differentiating photoreceptor cells, primarily R7, as well as cone cells [299];
- Lozenge-Gal4: leads to expression in cells of the “R7-equivalent group”: R7, R1/6 and cone cells [300];

- CG7077-Gal4: expression in pigment cells [301];
- Rh1-Gal4 (*ninaE*-Gal4): expression in photoreceptors R1-R6 [302].

Finally, the combination of the Gal4-UAS, high-throughput transgenesis, and RNA interference technologies has led to creation of a collection of transgenic fly lines carrying RNAi-constructs against most *Drosophila* genes under the control of the UAS-enhancer [303]. This collection (Vienna *Drosophila* RNAi Center, VDRC: <http://stockcenter.vdrc.at/control/main>) is a resource of tremendous value to researchers, as evidenced by the fact that since its appearance, VDRC has delivered more than six hundred thousand lines to different laboratories throughout the world.

EXAMPLES OF USAGE OF THE DROSOPHILA EYE AS A MODEL FOR STUDYING HUMAN DISEASES

Alzheimer's disease. In 2006 this disease affected about 20 million people. The disease is characterized by loss of neurons in the cerebral cortex and subcortical areas. In 1991, the "amyloid hypothesis" was formulated, according to which the underlying cause of the disease is deposition of neurotoxic amyloid β -peptide [304]. *Drosophila* is of a considerable interest as a model organism for studying Alzheimer's disease due to a large degree of homology and the interchangeability of human and *Drosophila* proteins. Thus, γ -secretase from flies properly cleaves the precursor of the human β -amyloid (APP). To create a complete model one had to express (mostly using the line GMR-Gal4) human APP and β -secretase in *Drosophila*, which led to formation of β -amyloid plaques and age-dependent neurodegeneration [305]. These phenotypes were restored by adding inhibitors of β -secretase and γ -secretase in fly food, proving the usefulness of this model in studying the mechanisms of the pathogenesis of Alzheimer's disease and in finding new means of its treatment [305].

There are also simpler models of Alzheimer's disease, directly expressing the β -amyloid peptide. Thus, expression of the peptide A β 42 in the *Drosophila* eye under the control of GMR-Gal4 led to defects in eye structure and neurodegeneration; the severity of the phenotypes depended on expression levels and age [306, 307]. While A β 42 forms stable aggregates in *Drosophila* tissues, the A β 40 peptide showed a lower stability and caused no phenotypes [306, 307]. The effectiveness of these models for drug discovery is shown by the example of restoration of the A β 42 phenotypes upon addition of the β -amyloid aggregation inhibitor Congo red to the fly food [307].

Parkinson's disease. The mechanism of this disease is not fully understood, but it is characterized by accumulation of α -synuclein aggregates and loss of dopamine

neurons in the human central nervous system [308]. Expression of human α -synuclein in *Drosophila* neurons (by *elav*-Gal4) and eye (by GMR-Gal4) led to accumulation of aggregates of the protein and neurodegeneration increasing with age [309]. The *Drosophila* model of Parkinson's disease was used to demonstrate for the first time the previously predicted relationship between α -synuclein phosphorylation and its ability to aggregate and induce neurodegeneration. Indeed, phosphorylation of α -synuclein on the site Ser129, previously observed in brain tissue of patients with Parkinson's disease, correlated with its aggregation in *Drosophila* [310]. Moreover, a non-phosphorylatable mutant form of α -synuclein (S129A) did not cause neurodegeneration in *Drosophila*, whereas the form S129D, simulating the constant phosphorylation, increased neurotoxicity in comparison to the wild-type form of α -synuclein [311].

Huntington's disease. This disease is caused by multiplication of the codon CAG (encoding glutamine) in the gene for the 350-kDa protein huntingtin [312]. Directed expression of the N-terminal fragment of human huntingtin with different numbers of glutamine repeats using GMR-Gal4 caused degeneration of photoreceptors; both the age at which degeneration began and its magnitude depended on the length of repeats [313]. It was also shown that proteins dTRP2 (homolog of the human TRP2 (tetratricopeptide repeat protein2)) and dHDJ1 (homolog of human HSP40/HDJ1) significantly rescued the phenotype, perhaps due to activation of the ATPase activity of hsp70 [314]. This assumption was confirmed by a study with directed expression of hsp70, which rescued the eye phenotypes [315]. Numerous investigations continue to study proteins genetically interacting with huntingtin in the *Drosophila* model to shed light on the mechanisms of Huntington's disease and hopefully contribute to future development of drugs against it.

The HumanaFly project. One of the important approaches to study cancer is to reproduce it in model organisms, including *Drosophila*. Signaling pathways that control differentiation, cell proliferation, and apoptosis are similar in all multicellular animals, and proteins involved in them are homologous and often interchangeable. As described in many examples above, disruption of these signaling pathways in the human body often leads to carcinogenesis. Knowledge about which gene causes these disorders permits identifying ways of blocking its activity, and therefore can be used to develop drugs. This is the basis of our project HumanaFly (Kryuchkov, Averkov, Khaustov, Katanaev, unpublished data). We use a cDNA plasmid library, prepared from mRNA from human breast cancer cells and cloned under the control of UAS-enhancer, to introduce human genes into the genome of *Drosophila* by high-throughput transgenesis. Overexpression of the transgene occurs in the developing eye of the insect using the GMR-Gal4 line. From numerous transgenes generated we select only those whose

expression leads to disruption of eye development, and operationally call them potential protooncogenes or tumor-suppressors. We identify these genes through sequencing. Analysis of morphology and histology of the adult eye and eye-antennal disc provides an initial idea of the mechanisms of developmental disorders caused by the human transgene. Subsequent genetic experiments examine the mechanism of pathogenesis and allow identifying the *Drosophila* signaling pathways or cellular programs that are affected by the human transgene. These studies and confirmation of the collected data in mammalian cells will help identify new proto-oncogenes and the mechanisms of their effect in carcinogenesis. The project HumanaFly represents the first large-scale attempt of using the developing *Drosophila* eye to study the effect of human proteins (potentially pathogenic) in the paths of intracellular signal transduction. The project is still in early stages, but we have already identified three potential new human protooncogenes, one of which unexpectedly affects the Wnt/Frizzled-signaling cascade.

In this review we discussed pathways of intracellular signal transduction that are active in animal development: the Notch, Hedgehog, TGF β , Wnt, PCP and the receptor tyrosine kinase pathways. Both insufficient activity and overactivation of these signaling pathways underlies many human diseases, especially cancer. Hopes for emergence of drugs against these diseases are associated with creation of adequate models for studying the underlying signaling cascades. The purpose of our review was to provide the developing eye of the fruit fly *Drosophila melanogaster* as such model. We have tried to describe in detail the development of the visual organ of *Drosophila* and the signaling pathways that regulate its various stages, in the hope that this model will be utilized by molecular and medical genetics looking for new approaches to study human diseases. The above-mentioned signaling pathways are used repeatedly in insect eye development, and defects (suppression of excessive activation) of any of them lead to clear morphological consequences. The high degree of homology and interchangeability of most protein components of these cascades between humans and *Drosophila* provide additional arguments for the utility of this model. Finally, precise genetic and histological techniques permit sophisticated experimentation on the fruit fly's eye, impossible with other model organisms. All these considerations argue for the active use of the developing *Drosophila* eye model to study the mechanisms of pathogenesis and for drug discovery. Indeed, a number of successful examples of such use in the case of neurodegenerative disease and cancer are mentioned in our article. We hope that future research will advance this success and that the fruit fly, after a hundred years of use for studying the fundamental laws of biology, will remain in demand as the object of research, this time also medical-ly oriented.

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REFERENCES

1. Wolff, T., and Ready, D. F. (1993) in *The Development of Drosophila melanogaster* (Bate, M., and Martinez Arias, A., eds.) Cold Spring Harbor Laboratory Press, pp. 1277-1325.
2. Kryuchkov, M., Katanaev, V. L., Enin, G. A., Sergeev, A., Timchenko, A. A., and Serdyuk, I. N. (2011) *PLoS ONE*, **6**, e22237.
3. Bernhard, C. G., Gemne, G., and Sallstrom, J. (1970) *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.*, **67**, 1-25.
4. Miller, W. H., Bernard, G. D., and Allen, J. L. (1968) *Science*, **162**, 760-767.
5. Palasantzas, G., de Hosson, J. T. M., Michielsen, K. F. L., and Stavenga, D. G. (2005) in *Handbook of Nanostructured Biomaterials and Their Applications in Nanobiotechnology* (Nalwa, H. S., ed.) American Scientific Publishers, pp. 273-301.
6. Kirschfeld, K., Feiler, R., and Franceschini, N. (1978) *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.*, **125**, 275-284.
7. Salcedo, E., Huber, A., Henrich, S., Chadwell, L. V., Chou, W. H., Paulsen, R., and Britt, S. G. (1999) *J. Neurosci.*, **19**, 10716-10726.
8. Papatsenko, D., Sheng, G., and Desplan, C. (1997) *Development*, **124**, 1665-1673.
9. Tomlinson, A. (2003) *Dev. Cell*, **5**, 799-809.
10. Quiring, R., Walldorf, U., Kloter, U., and Gehring, W. J. (1994) *Science*, **265**, 785-789.
11. Halder, G., Callaerts, P., and Gehring, W. J. (1995) *Science*, **267**, 1788-1792.
12. Tomlinson, A., and Struhl, G. (2001) *Mol. Cell*, **7**, 487-495.
13. Cayouette, M., and Raff, M. (2002) *Nat. Neurosci.*, **5**, 1265-1269.
14. Janes, K. A., and Yaffe, M. B. (2006) *Nat. Rev. Mol. Cell Biol.*, **7**, 820-828.
15. Kiel, C., Yus, E., and Serrano, L. (2010) *Cell*, **140**, 33-47.
16. Gerhart, J. (1999) *Teratology*, **60**, 226-239.
17. Levitzki, A., and Klein, S. (2010) *Mol. Aspects Med.*, **31**, 287-329.
18. Artavanis-Tsakonas, S., and Muskavitch, M. A. (2010) *Curr. Top. Dev. Biol.*, **92**, 1-29.
19. Ehebauer, M., Hayward, P., and Martinez-Arias, A. (2006) *Sci. STKE*, **2006**, cm7.
20. Wharton, K. A., Johansen, K. M., Xu, T., and Artavanis-Tsakonas, S. (1985) *Cell*, **43**, 567-581.
21. Rebay, I., Fleming, R. J., Fehon, R. G., Cherbas, L., Cherbas, P., and Artavanis-Tsakonas, S. (1991) *Cell*, **67**, 687-699.
22. Aster, J. C., Pear, W. S., and Blacklow, S. C. (2008) *Annu. Rev. Physiol.*, **3**, 587-613.
23. Stanley, P., and Okajima, T. (2010) *Curr. Top. Dev. Biol.*, **92**, 131-164.

24. Blaumueller, C. M., Qi, H., Zagouras, P., and Artavanis-Tsakonas, S. (1997) *Cell*, **90**, 281-291.
25. Logeat, F., Bessia, C., Brou, C., LeBail, O., Jarriault, S., Seidah, N. G., and Israel, A. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 8108-8112.
26. Zolkiewska, A. (2008) *Cell. Mol. Life Sci.*, **65**, 2056-2068.
27. Jorissen, E., and de Strooper, B. (2010) *Curr. Top. Dev. Biol.*, **92**, 201-230.
28. Struhl, G., and Adachi, A. (1998) *Cell*, **93**, 649-660.
29. Bray, S., and Bernard, F. (2010) *Curr. Top. Dev. Biol.*, **92**, 253-275.
30. Krejci, A., Bernard, F., Housden, B. E., Collins, S., and Bray, S. J. (2009) *Sci. Signal.*, **2**, ra1.
31. Heitzler, P., Bourouis, M., Ruel, L., Carteret, C., and Simpson, P. (1996) *Development*, **122**, 161-171.
32. Sprinzak, D., Lakhapal, A., Lebon, L., Santat, L. A., Fontes, M. E., Anderson, G. A., Garcia-Ojalvo, J., and Elowitz, M. B. (2010) *Nature*, **465**, 86-90.
33. Barad, O., Rosin, D., Hornstein, E., and Barkai, N. (2010) *Sci. Signal.*, **3**, ra51.
34. Bray, S. (1998) *Semin. Cell Dev. Biol.*, **9**, 591-597.
35. Ellisen, L. W., Bird, J., West, D. C., Soreng, A. L., Reynolds, T. C., Smith, S. D., and Sklar, J. (1991) *Cell*, **66**, 649-661.
36. Weng, A. P., Ferrando, A. A., Lee, W., Morris, J. P. T., Silverman, L. B., Sanchez-Irizarry, C., Blacklow, S. C., Look, A. T., and Aster, J. C. (2004) *Science*, **306**, 269-271.
37. Malecki, M. J., Sanchez-Irizarry, C., Mitchell, J. L., Histen, G., Xu, M. L., Aster, J. C., and Blacklow, S. C. (2006) *Mol. Cell Biol.*, **26**, 4642-4651.
38. Tang, S. C., Jeng, J. S., Lee, M. J., and Yip, P. K. (2009) *Acta Neurol. Taiwan*, **18**, 81-90.
39. Tournier-Lasserre, E., Joutel, A., Melki, J., Weissenbach, J., Lathrop, G. M., Chabriot, H., Mas, J. L., Cabanis, E. A., Baudrimont, M., Maciazek, J., et al. (1993) *Nat. Genet.*, **3**, 256-259.
40. Joutel, A., Corpechot, C., Ducros, A., Vahedi, K., Chabriot, H., Mouton, P., Alamowitch, S., Domenga, V., Cecillon, M., Marechal, E., Maciazek, J., Vayssiere, C., Cruaud, C., Cabanis, E. A., Ruchoux, M. M., Weissenbach, J., Bach, J. F., Bousser, M. G., and Tournier-Lasserre, E. (1996) *Nature*, **383**, 707-710.
41. Joutel, A., Vahedi, K., Corpechot, C., Troesch, A., Chabriot, H., Vayssiere, C., Cruaud, C., Maciazek, J., Weissenbach, J., Bousser, M. G., Bach, J. F., and Tournier-Lasserre, E. (1997) *Lancet*, **350**, 1511-1515.
42. Harper, J. A., Yuan, J. S., Tan, J. B., Visan, I., and Guidos, C. J. (2003) *Clin. Genet.*, **64**, 461-472.
43. Joutel, A., Andreux, F., Gaulis, S., Domenga, V., Cecillon, M., Battail, N., Piga, N., Chapon, F., Godfrain, C., and Tournier-Lasserre, E. (2000) *J. Clin. Invest.*, **105**, 597-605.
44. Opherck, C., Duering, M., Peters, N., Karpinska, A., Rosner, S., Schneider, E., Bader, B., Giese, A., and Dichgans, M. (2009) *Hum. Mol. Genet.*, **18**, 2761-2767.
45. Krantz, I. D., Piccoli, D. A., and Spinner, N. B. (1999) *Curr. Opin. Pediatr.*, **11**, 558-564.
46. Oda, T., Elkahoul, A. G., Pike, B. L., Okajima, K., Krantz, I. D., Genin, A., Piccoli, D. A., Meltzer, P. S., Spinner, N. B., Collins, F. S., and Chandrasekharappa, S. C. (1997) *Nat. Genet.*, **16**, 235-242.
47. Li, L., Krantz, I. D., Deng, Y., Genin, A., Banta, A. B., Collins, C. C., Qi, M., Trask, B. J., Kuo, W. L., Cochran, J., Costa, T., Pierpont, M. E., Rand, E. B., Piccoli, D. A., Hood, L., and Spinner, N. B. (1997) *Nat. Genet.*, **16**, 243-251.
48. McDaniel, R., Warthen, D. M., Sanchez-Lara, P. A., Pai, A., Krantz, I. D., Piccoli, D. A., and Spinner, N. B. (2006) *Am. J. Hum. Genet.*, **79**, 169-173.
49. Talora, C., Campese, A. F., Bellavia, D., Felli, M. P., Vacca, A., Gulino, A., and Screpanti, I. (2008) *Biochim. Biophys. Acta*, **1782**, 489-497.
50. High, F. A., and Epstein, J. A. (2008) *Nat. Rev. Genet.*, **9**, 49-61.
51. Papayannopoulos, V., Tomlinson, A., Panin, V. M., Rauskolb, C., and Irvine, K. D. (1998) *Science*, **281**, 2031-2034.
52. Dominguez, M., and de Celis, J. F. (1998) *Nature*, **396**, 276-278.
53. Cho, K. O., and Choi, K. W. (1998) *Nature*, **396**, 272-276.
54. Chao, J. L., Tsai, Y. C., Chiu, S. J., and Sun, Y. H. (2004) *Development*, **131**, 3839-3847.
55. Frankfort, B. J., and Mardon, G. (2002) *Development*, **129**, 1295-1306.
56. Baonza, A., and Freeman, M. (2001) *Development*, **128**, 3889-3898.
57. Baker, N. E., and Yu, S. Y. (1997) *Curr. Biol.*, **7**, 122-132.
58. Cagan, R. L., and Ready, D. F. (1989) *Genes Dev.*, **3**, 1099-1112.
59. Sun, Y., Jan, L. Y., and Jan, Y. N. (1998) *Development*, **125**, 3731-3740.
60. Van Doren, M., Powell, P. A., Pasternak, D., Singson, A., and Posakony, J. W. (1992) *Genes Dev.*, **6**, 2592-2605.
61. Cooper, M. T., and Bray, S. J. (2000) *Curr. Biol.*, **10**, 1507-1510.
62. Miller, A. C., Lyons, E. L., and Herman, T. G. (2009) *Curr. Biol.*, **19**, 1378-1383.
63. Rhyu, M. S., Jan, L. Y., and Jan, Y. N. (1994) *Cell*, **76**, 477-491.
64. Frise, E., Knoblich, J. A., Younger-Shepherd, S., Jan, L. Y., and Jan, Y. N. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 11925-11932.
65. Le Borgne, R., and Schweisguth, F. (2003) *Dev. Cell*, **5**, 139-148.
66. Fichelson, P., and Ghosh, M. (2003) *Development*, **130**, 123-133.
67. Nusslein-Volhard, C., and Wieschaus, E. (1980) *Nature*, **287**, 795-801.
68. Porter, J. A., Ekker, S. C., Park, W. J., von Kessler, D. P., Young, K. E., Chen, C. H., Ma, Y., Woods, A. S., Cotter, R. J., Koonin, E. V., and Beachy, P. A. (1996) *Cell*, **86**, 21-34.
69. Porter, J. A., Young, K. E., and Beachy, P. A. (1996) *Science*, **274**, 255-259.
70. Koonin, E. V. (1995) *Trends Biochem. Sci.*, **20**, 141-142.
71. Chamoun, Z., Mann, R. K., Nellen, D., von Kessler, D. P., Bellotto, M., Beachy, P. A., and Basler, K. (2001) *Science*, **293**, 2080-2084.
72. Burke, R., Nellen, D., Bellotto, M., Hafen, E., Senti, K. A., Dickson, B. J., and Basler, K. (1999) *Cell*, **99**, 803-815.
73. Lee, J. J., Ekker, S. C., von Kessler, D. P., Porter, J. A., Sun, B. I., and Beachy, P. A. (1994) *Science*, **266**, 1528-1537.
74. Lawrence, P. A. (2001) *Nat. Cell Biol.*, **3**, E151-154.

75. Zeng, X., Goetz, J. A., Suber, L. M., Scott, W. J., Jr., Schreiner, C. M., and Robbins, D. J. (2001) *Nature*, **411**, 716-720.
76. Panakova, D., Sprong, H., Marois, E., Thiele, C., and Eaton, S. (2005) *Nature*, **435**, 58-65.
77. Katanaev, V. L., Solis, G. P., Hausmann, G., Buestorf, S., Katanayeva, N., Schrock, Y., Stuermer, C. A., and Basler, K. (2008) *EMBO J.*, **27**, 509-521.
78. Rietveld, A., Neutz, S., Simons, K., and Eaton, S. (1999) *J. Biol. Chem.*, **274**, 12049-12054.
79. Ingham, P. W., Taylor, A. M., and Nakano, Y. (1991) *Nature*, **353**, 184-187.
80. Marigo, V., Davey, R. A., Zuo, Y., Cunningham, J. M., and Tabin, C. J. (1996) *Nature*, **384**, 176-179.
81. Zheng, X., Mann, R. K., Sever, N., and Beachy, P. A. (2010) *Genes Dev.*, **24**, 57-71.
82. Alcedo, J., Ayzenzon, M., von Ohlen, T., Noll, M., and Hooper, J. E. (1996) *Cell*, **86**, 221-232.
83. Taipale, J., Cooper, M. K., Maiti, T., and Beachy, P. A. (2002) *Nature*, **418**, 892-897.
84. Riobo, N. A., Saucy, B., Dilizio, C., and Manning, D. R. (2006) *Proc. Natl. Acad. Sci. USA*, **103**, 12607-12612.
85. Robbins, D. J., Nybakken, K. E., Kobayashi, R., Sisson, J. C., Bishop, J. M., and Therond, P. P. (1997) *Cell*, **90**, 225-234.
86. Sisson, J. C., Ho, K. S., Suyama, K., and Scott, M. P. (1997) *Cell*, **90**, 235-245.
87. Ingham, P. W., Nakano, Y., and Seger, C. (2011) *Nat. Rev. Genet.*, **12**, 393-406.
88. Jia, J., Zhang, L., Zhang, Q., Tong, C., Wang, B., Hou, F., Amanai, K., and Jiang, J. (2005) *Dev. Cell*, **9**, 819-830.
89. Mullor, J. L., Sanchez, P., and Ruiz i Altaba, A. (2002) *Trends Cell Biol.*, **12**, 562-569.
90. Bale, A. E. (2002) *Annu. Rev. Genom. Hum. Genet.*, **3**, 47-65.
91. Belloni, E., Muenke, M., Roessler, E., Traverso, G., Siegel-Bartelt, J., Frumkin, A., Mitchell, H. F., Donis-Keller, H., Helms, C., Hing, A. V., Heng, H. H., Koop, B., Martindale, D., Rommens, J. M., Tsui, L. C., and Scherer, S. W. (1996) *Nat. Genet.*, **14**, 353-356.
92. Roessler, E., Belloni, E., Gaudenz, K., Jay, P., Berta, P., Scherer, S. W., Tsui, L. C., and Muenke, M. (1996) *Nat. Genet.*, **14**, 357-360.
93. Ming, J. E., Kaupas, M. E., Roessler, E., Brunner, H. G., Golabi, M., Tekin, M., Stratton, R. F., Sujansky, E., Bale, S. J., and Muenke, M. (2002) *Hum. Genet.*, **110**, 297-301.
94. Binns, W., James, L. F., Shupe, J. L., and Everett, G. (1963) *Am. J. Vet. Res.*, **24**, 1164-1175.
95. Keeler, R. F., and Binns, W. (1968) *Teratology*, **1**, 5-10.
96. Chen, J. K., Taipale, J., Cooper, M. K., and Beachy, P. A. (2002) *Genes Dev.*, **16**, 2743-2748.
97. Epstein, E. H. (2008) *Nat. Rev. Cancer*, **8**, 743-754.
98. Xie, J. (2008) *Acta Biochim. Biophys. Sin. (Shanghai)*, **40**, 670-680.
99. Rubin, L. L., and de Sauvage, F. J. (2006) *Nat. Rev. Drug Discov.*, **5**, 1026-1033.
100. Roignant, J. Y., and Treisman, J. E. (2009) *Int. J. Dev. Biol.*, **53**, 795-804.
101. Heberlein, U., Singh, C. M., Luk, A. Y., and Donohoe, T. J. (1995) *Nature*, **373**, 709-711.
102. Heberlein, U., Wolff, T., and Rubin, G. M. (1993) *Cell*, **75**, 913-926.
103. Ma, C., Zhou, Y., Beachy, P. A., and Moses, K. (1993) *Cell*, **75**, 927-938.
104. Dominguez, M., and Hafen, E. (1997) *Genes Dev.*, **11**, 3254-3264.
105. Ou, C. Y., Lin, Y. F., Chen, Y. J., and Chien, C. T. (2002) *Genes Dev.*, **16**, 2403-2414.
106. Gray, A. M., and Mason, A. J. (1990) *Science*, **247**, 1328-1330.
107. Massague, J. (2000) *Nat. Rev. Mol. Cell Biol.*, **1**, 169-178.
108. Raftery, L. A., and Sutherland, D. J. (1999) *Dev. Biol.*, **210**, 251-268.
109. Nellen, D., Affolter, M., and Basler, K. (1994) *Cell*, **78**, 225-237.
110. Brummel, T. J., Twombly, V., Marques, G., Wrana, J. L., Newfeld, S. J., Attisano, L., Massague, J., O'Connor, M. B., and Gelbart, W. M. (1994) *Cell*, **78**, 251-261.
111. Letsou, A., Arora, K., Wrana, J. L., Simin, K., Twombly, V., Jamal, J., Staehling-Hampton, K., Hoffmann, F. M., Gelbart, W. M., Massague, J., et al. (1995) *Cell*, **80**, 899-908.
112. Ruberte, E., Marty, T., Nellen, D., Affolter, M., and Basler, K. (1995) *Cell*, **80**, 889-897.
113. Sekelsky, J. J., Newfeld, S. J., Raftery, L. A., Chartoff, E. H., and Gelbart, W. M. (1995) *Genetics*, **139**, 1347-1358.
114. Hudson, J. B., Podos, S. D., Keith, K., Simpson, S. L., and Ferguson, E. L. (1998) *Development*, **125**, 1407-1420.
115. Wisotzkey, R. G., Mehra, A., Sutherland, D. J., Dobens, L. L., Liu, X., Dohrmann, C., Attisano, L., and Raftery, L. A. (1998) *Development*, **125**, 1433-1445.
116. Das, P., Maduzia, L. L., Wang, H., Finelli, A. L., Cho, S. H., Smith, M. M., and Padgett, R. W. (1998) *Development*, **125**, 1519-1528.
117. Harradine, K. A., and Akhurst, R. J. (2006) *Ann. Med.*, **38**, 403-414.
118. Lebreiro, A., Martins, E., Cruz, C., Almeida, J., Maciel, M. J., Cardoso, J. C., and Lima, C. A. (2010) *Rev. Port. Cardiol.*, **29**, 1021-1036.
119. Neptune, E. R., Frischmeyer, P. A., Arking, D. E., Myers, L., Bunton, T. E., Gayraud, B., Ramirez, F., Sakai, L. Y., and Dietz, H. C. (2003) *Nat. Genet.*, **33**, 407-411.
120. Meulmeester, E., and Ten Dijke, P. (2010) *J. Pathol.*, **223**, 205-218.
121. Hahn, S. A., Schutte, M., Hoque, A. T., Moskaluk, C. A., da Costa, L. T., Rozenblum, E., Weinstein, C. L., Fischer, A., Yeo, C. J., Hruban, R. H., and Kern, S. E. (1996) *Science*, **271**, 350-353.
122. Sonis, S. T., van Vugt, A. G., Brien, J. P., Muska, A. D., Bruskin, A. M., Rose, A., and Haley, J. D. (1997) *Oral Oncol.*, **33**, 47-54.
123. Hannon, G. J., and Beach, D. (1994) *Nature*, **371**, 257-261.
124. Datto, M. B., Li, Y., Panus, J. F., Howe, D. J., Xiong, Y., and Wang, X. F. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 5545-5549.
125. Coffey, R. J., Jr., Bascom, C. C., Sipes, N. J., Graves-Deal, R., Weissman, B. E., and Moses, H. L. (1988) *Mol. Cell. Biol.*, **8**, 3088-3093.
126. Oberhammer, F. A., Pavelka, M., Sharma, S., Tiefenbacher, R., Purchio, A. F., Bursch, W., and Schulte-Hermann, R. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 5408-5412.
127. Drabsch, Y., and ten Dijke, P. (2011) *J. Mammary Gland Biol. Neoplasia*, **16**, 97-108.

128. Parvani, J. G., Taylor, M. A., and Schiemann, W. P. (2011) *J. Mammary Gland Biol. Neoplasia*, **16**, 127-146.
129. Tabata, T., and Takei, Y. (2004) *Development*, **131**, 703-712.
130. Bessa, J., Gebelein, B., Pichaud, F., Casares, F., and Mann, R. S. (2002) *Genes Dev.*, **16**, 2415-2427.
131. Greenwood, S., and Struhl, G. (1999) *Development*, **126**, 5795-5808.
132. Curtiss, J., and Mlodzik, M. (2000) *Development*, **127**, 1325-1336.
133. Logan, C. Y., and Nusse, R. (2004) *Annu. Rev. Cell Dev. Biol.*, **20**, 781-810.
134. Cabrera, C. V., Alonso, M. C., Johnston, P., Phillips, R. G., and Lawrence, P. A. (1987) *Cell*, **50**, 659-663.
135. Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigel, D., and Nusse, R. (1987) *Cell*, **50**, 649-657.
136. Coudreuse, D., and Korswagen, H. C. (2007) *Development*, **134**, 3-12.
137. Port, F., and Basler, K. (2010) *Traffic*, **11**, 1265-1271.
138. Tanaka, K., Kitagawa, Y., and Kadowaki, T. (2002) *J. Biol. Chem.*, **277**, 12816-12823.
139. Willert, K., Brown, J. D., Danenberg, E., Duncan, A. W., Weissman, I. L., Reya, T., Yates, J. R., 3rd, and Nusse, R. (2003) *Nature*, **423**, 448-452.
140. Kadowaki, T., Wilder, E., Klingensmith, J., Zachary, K., and Perrimon, N. (1996) *Genes Dev.*, **10**, 3116-3128.
141. Van den Heuvel, M., Harryman-Samos, C., Klingensmith, J., Perrimon, N., and Nusse, R. (1993) *EMBO J.*, **12**, 5293-5302.
142. Takada, R., Satomi, Y., Kurata, T., Ueno, N., Norioka, S., Kondoh, H., Takao, T., and Takada, S. (2006) *Dev. Cell*, **11**, 791-801.
143. Banziger, C., Soldini, D., Schutt, C., Zipperlen, P., Hausmann, G., and Basler, K. (2006) *Cell*, **125**, 509-522.
144. Bartscherer, K., Pelte, N., Ingelfinger, D., and Boutros, M. (2006) *Cell*, **125**, 523-533.
145. Goodman, R. M., Thombre, S., Firtina, Z., Gray, D., Betts, D., Roebuck, J., Spana, E. P., and Selva, E. M. (2006) *Development*, **133**, 4901-4911.
146. Coudreuse, D. Y., Roel, G., Betist, M. C., Destree, O., and Korswagen, H. C. (2006) *Science*, **312**, 921-924.
147. Franch-Marro, X., Wendler, F., Guidato, S., Griffith, J., Baena-Lopez, A., Itasaki, N., Maurice, M. M., and Vincent, J. P. (2008) *Nat. Cell Biol.*, **10**, 170-177.
148. Port, F., Kuster, M., Herr, P., Furger, E., Banziger, C., Hausmann, G., and Basler, K. (2008) *Nat. Cell Biol.*, **10**, 178-185.
149. Belenkaya, T. Y., Wu, Y., Tang, X., Zhou, B., Cheng, L., Sharma, Y. V., Yan, D., Selva, E. M., and Lin, X. (2008) *Dev. Cell*, **14**, 120-131.
150. Papkoff, J., and Schryver, B. (1990) *Mol. Cell. Biol.*, **10**, 2723-2730.
151. Egger-Adam, D., and Katanaev, V. L. (2008) *Front. Biosci.*, **13**, 4740-4755.
152. Katanaev, V. L. (2010) *Biochemistry (Moscow)*, **75**, 1428-1434.
153. Koval, A., Purvanov, V., Egger-Adam, D., and Katanaev, V. L. (2011) *Biochem. Pharmacol.*, **82**, 1311-1319.
154. Pierce, K. L., Premont, R. T., and Lefkowitz, R. J. (2002) *Nat. Rev. Mol. Cell Biol.*, **3**, 639-650.
155. Gilman, A. G. (1987) *Annu. Rev. Biochem.*, **56**, 615-649.
156. Katanaev, V. L., Ponzielli, R., Semeriva, M., and Tomlinson, A. (2005) *Cell*, **120**, 111-122.
157. Koval, A., and Katanaev, V. L. (2011) *Biochem. J.*, **433**, 435-440.
158. Gao, C., and Chen, Y. G. (2010) *Cell. Signal.*, **22**, 717-727.
159. Umbhauer, M., Djiane, A., Goisset, C., Penzo-Mendez, A., Riou, J. F., Boucaut, J. C., and Shi, D. L. (2000) *EMBO J.*, **19**, 4944-4954.
160. Oldham, W. M., and Hamm, H. E. (2007) *Adv. Protein Chem.*, **74**, 67-93.
161. Bikkavilli, R. K., Feigin, M. E., and Malbon, C. C. (2008) *J. Cell Sci.*, **121**, 234-245.
162. Angers, S., Thorpe, C. J., Biechele, T. L., Goldenberg, S. J., Zheng, N., MacCoss, M. J., and Moon, R. T. (2006) *Nat. Cell Biol.*, **8**, 348-357.
163. Egger-Adam, D., and Katanaev, V. L. (2010) *Dev. Dyn.*, **239**, 168-183.
164. Jung, H., Kim, H. J., Lee, S. K., Kim, R., Kopachik, W., Han, J. K., and Jho, E. H. (2009) *Exp. Mol. Med.*, **41**, 695-706.
165. Wedegaertner, P. B., Wilson, P. T., and Bourne, H. R. (1995) *J. Biol. Chem.*, **270**, 503-506.
166. Kimelman, D., and Xu, W. (2006) *Oncogene*, **25**, 7482-7491.
167. Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. (1997) *EMBO J.*, **16**, 3797-3804.
168. Cortese, M. S., Uversky, V. N., and Dunker, A. K. (2008) *Prog. Biophys. Mol. Biol.*, **98**, 85-106.
169. Noutsou, M., Duarte, A. M., Anvarian, Z., Didenko, T., Minde, D. P., Kuper, I., de Ridder, I., Oikonomou, C., Friedler, A., Boelens, R., Rudiger, S. G., and Maurice, M. M. (2011) *J. Mol. Biol.*, **405**, 773-786.
170. Spink, K. E., Polakis, P., and Weis, W. I. (2000) *EMBO J.*, **19**, 2270-2279.
171. Kishida, S., Yamamoto, H., Hino, S., Ikeda, S., Kishida, M., and Kikuchi, A. (1999) *Mol. Cell. Biol.*, **19**, 4414-4422.
172. Cliffe, A., Hamada, F., and Bienz, M. (2003) *Curr. Biol.*, **13**, 960-966.
173. Mao, J., Wang, J., Liu, B., Pan, W., Farr, G. H., 3rd, Flynn, C., Yuan, H., Takada, S., Kimelman, D., Li, L., and Wu, D. (2001) *Mol. Cell*, **7**, 801-809.
174. Davidson, G., Wu, W., Shen, J., Bilic, J., Fenger, U., Stanek, P., Glinka, A., and Niehrs, C. (2005) *Nature*, **438**, 867-872.
175. Zeng, X., Tamai, K., Doble, B., Li, S., Huang, H., Habas, R., Okamura, H., Woodgett, J., and He, X. (2005) *Nature*, **438**, 873-877.
176. Willert, K., and Jones, K. A. (2006) *Genes Dev.*, **20**, 1394-1404.
177. Seto, E. S., and Bellen, H. J. (2006) *J. Cell Biol.*, **173**, 95-106.
178. Purvanov, V., Koval, A., and Katanaev, V. L. (2010) *Sci. Signal.*, **3**, ra65.
179. Moon, R. T., Kohn, A. D., de Ferrari, G. V., and Kaykas, A. (2004) *Nat. Rev. Genet.*, **5**, 691-701.
180. Little, R. D., Carulli, J. P., Del Mastro, R. G., Dupuis, J., Osborne, M., Folz, C., Manning, S. P., Swain, P. M., Zhao, S. C., Eustace, B., Lappe, M. M., Spitzer, L., Zweier, S., Braunschweiger, K., Benchekroun, Y., Hu, X., Adair, R., Chee, L., FitzGerald, M. G., Tulig, C., Caruso, A., Tzellas, N., Bawa, A., Franklin, B., McGuire, S., Nogues, X., Gong, G., Allen, K. M., Anisowicz, A., Morales, A. J., Lomedico, P. T., Recker, S. M., van Eerdewegh, P., Recker, R. R., and Johnson, M. L. (2002) *Am. J. Hum. Genet.*, **70**, 11-19.

181. Boyden, L. M., Mao, J., Belsky, J., Mitzner, L., Farhi, A., Mitnick, M. A., Wu, D., Insogna, K., and Lifton, R. P. (2002) *N. Engl. J. Med.*, **346**, 1513-1521.
182. Gong, Y., Slee, R. B., Fukai, N., Rawadi, G., Roman-Roman, S., Reginato, A. M., Wang, H., Cundy, T., Glorieux, F. H., Lev, D., Zacharin, M., Oexle, K., Marcelino, J., Suwairi, W., Heeger, S., Sabatakos, G., Apte, S., Adkins, W. N., Allgrove, J., Arslan-Kirchner, M., Batch, J. A., Beighton, P., Black, G. C., Boles, R. G., Boon, L. M., Borrone, C., Brunner, H. G., Carle, G. F., Dallapiccola, B., de Paepe, A., Floege, B., Halfhide, M. L., Hall, B., Hennekam, R. C., Hirose, T., Jans, A., Juppner, H., Kim, C. A., Keppler-Noreuil, K., Kohlschuetter, A., LaCombe, D., Lambert, M., Lemyre, E., Letteboer, T., Peltonen, L., Ramesar, R. S., Romanengo, M., Somer, H., Steichen-Gersdorf, E., Steinmann, B., Sullivan, B., Superti-Furga, A., Swoboda, W., van den Boogaard, M. J., van Hul, W., Vikkula, M., Votruba, M., Zabel, B., Garcia, T., Baron, R., Olsen, B. R., and Warman, M. L. (2001) *Cell*, **107**, 513-523.
183. Robitaille, J., MacDonald, M. L., Kaykas, A., Sheldahl, L. C., Zeisler, J., Dube, M. P., Zhang, L. H., Singaraja, R. R., Guernsey, D. L., Zheng, B., Siebert, L. F., Hoskin-Mott, A., Trese, M. T., Pimstone, S. N., Shastry, B. S., Moon, R. T., Hayden, M. R., Goldberg, Y. P., and Samuels, M. E. (2002) *Nat. Genet.*, **32**, 326-330.
184. Kaykas, A., Yang-Snyder, J., Heroux, M., Shah, K. V., Bouvier, M., and Moon, R. T. (2004) *Nat. Cell Biol.*, **6**, 52-58.
185. Polakis, P. (2007) *Curr. Opin. Genet. Dev.*, **17**, 45-51.
186. Turashvili, G., Bouchal, J., Burkadze, G., and Kolar, Z. (2006) *Pathobiology*, **73**, 213-223.
187. Giles, R. H., van Es, J. H., and Clevers, H. (2003) *Biochim. Biophys. Acta*, **1653**, 1-24.
188. Zhao, J., Kim, K. A., and Abo, A. (2009) *Trends Biotechnol.*, **27**, 131-136.
189. Minear, S., Leucht, P., Jiang, J., Liu, B., Zeng, A., Fuerer, C., Nüsse, R., and Helms, J. A. (2010) *Sci. Transl. Med.*, **2**, 29ra30.
190. Van de Schans, V. A., Smits, J. F., and Blankesteyn, W. M. (2008) *Eur. J. Pharmacol.*, **585**, 338-345.
191. Budnik, V., and Salinas, P. C. (2011) *Curr. Opin. Neurobiol.*, **21**, 151-159.
192. De Ferrari, G. V., and Moon, R. T. (2006) *Oncogene*, **25**, 7545-7553.
193. Magdesian, M. H., Carvalho, M. M., Mendes, F. A., Saraiva, L. M., Juliano, M. A., Juliano, L., Garcia-Abreu, J., and Ferreira, S. T. (2008) *J. Biol. Chem.*, **283**, 9359-9368.
194. Alvarez, A. R., Godoy, J. A., Mullendorff, K., Olivares, G. H., Bronfman, M., and Inestrosa, N. C. (2004) *Exp. Cell Res.*, **297**, 186-196.
195. Cerpa, W., Toledo, E. M., Varela-Nallar, L., and Inestrosa, N. C. (2009) *Drug News Perspect.*, **22**, 579-591.
196. Angers, S., and Moon, R. T. (2009) *Nat. Rev. Mol. Cell Biol.*, **10**, 468-477.
197. Barker, N., and Clevers, H. (2006) *Nat. Rev. Drug Discov.*, **5**, 997-1014.
198. Legent, K., and Treisman, J. E. (2008) *Methods Mol. Biol.*, **469**, 141-161.
199. Cho, K. O., Chern, J., Izaddoost, S., and Choi, K. W. (2000) *Cell*, **103**, 331-342.
200. Heberlein, U., Borod, E. R., and Chanut, F. A. (1998) *Development*, **125**, 567-577.
201. Royet, J., and Finkelstein, R. (1997) *Development*, **124**, 4793-4800.
202. Baker, N. E. (1988) *Development*, **102**, 489-497.
203. Royet, J., and Finkelstein, R. (1996) *Development*, **122**, 1849-1858.
204. Baonza, A., and Freeman, M. (2002) *Development*, **129**, 5313-5322.
205. Treier, M., Bohmann, D., and Mlodzik, M. (1995) *Cell*, **83**, 753-760.
206. Treisman, J. E., and Rubin, G. M. (1995) *Development*, **121**, 3519-3527.
207. Fortini, M. E., and Rubin, G. M. (1991) *Cell Tissue Res.*, **265**, 185-191.
208. Wernet, M. F., Labhart, T., Baumann, F., Mazzoni, E. O., Pichaud, F., and Desplan, C. (2003) *Cell*, **115**, 267-279.
209. Lin, H. V., Rogulja, A., and Cadigan, K. M. (2004) *Development*, **131**, 2409-2418.
210. Cordero, J., Jassim, O., Bao, S., and Cagan, R. (2004) *Mech. Dev.*, **121**, 1523-1530.
211. Lim, H. Y., and Tomlinson, A. (2006) *Development*, **133**, 3529-3537.
212. Brunner, E., Brunner, D., Fu, W., Hafen, E., and Basler, K. (1999) *Dev. Biol.*, **206**, 178-188.
213. Tomlinson, A., Strapps, W. R., and Heemskerk, J. (1997) *Development*, **124**, 4515-4521.
214. Wehrli, M., and Tomlinson, A. (1998) *Development*, **125**, 1421-1432.
215. Tomlinson, A., and Struhl, G. (1999) *Development*, **126**, 5725-5738.
216. Veeman, M. T., Axelrod, J. D., and Moon, R. T. (2003) *Dev. Cell*, **5**, 367-377.
217. Simons, M., and Mlodzik, M. (2008) *Annu. Rev. Genet.*, **42**, 517-540.
218. Katanaev, V. L., and Tomlinson, A. (2006) *Cell Cycle*, **5**, 2464-2472.
219. Katanaev, V. L. (2001) *Biochemistry (Moscow)*, **66**, 351-368.
220. Vinson, C. R., Conover, S., and Adler, P. N. (1989) *Nature*, **338**, 263-264.
221. Moon, R. T., Campbell, R. M., Christian, J. L., McGrew, L. L., Shih, J., and Fraser, S. (1993) *Development*, **119**, 97-111.
222. Heisenberg, C. P., Tada, M., Rauch, G. J., Saude, L., Concha, M. L., Geisler, R., Stemple, D. L., Smith, J. C., and Wilson, S. W. (2000) *Nature*, **405**, 76-81.
223. Struhl, G., Barbash, D. A., and Lawrence, P. A. (1997) *Development*, **124**, 2155-2165.
224. Lawrence, P. A., Casal, J., and Struhl, G. (2002) *Development*, **129**, 2749-2760.
225. Yang, C. H., Axelrod, J. D., and Simon, M. A. (2002) *Cell*, **108**, 675-688.
226. Ma, D., Yang, C. H., McNeill, H., Simon, M. A., and Axelrod, J. D. (2003) *Nature*, **421**, 543-547.
227. Casal, J., Lawrence, P. A., and Struhl, G. (2006) *Development*, **133**, 4561-4572.
228. Strutt, D. I. (2001) *Mol. Cell*, **7**, 367-375.
229. Le Garrec, J. F., Lopez, P., and Kerszberg, M. (2006) *Dev. Dyn.*, **235**, 235-246.
230. Krasnow, R. E., Wong, L. L., and Adler, P. N. (1995) *Development*, **121**, 4095-4102.

231. Gubb, D., and Garcia-Bellido, A. (1982) *J. Embryol. Exp. Morphol.*, **68**, 37-57.
232. Wolff, T., and Rubin, G. M. (1998) *Development*, **125**, 1149-1159.
233. Taylor, J., Abramova, N., Charlton, J., and Adler, P. N. (1998) *Genetics*, **150**, 199-210.
234. Feiguin, F., Hannus, M., Mlodzik, M., and Eaton, S. (2001) *Dev. Cell*, **1**, 93-101.
235. Lu, B., Usui, T., Uemura, T., Jan, L., and Jan, Y. N. (1999) *Curr. Biol.*, **9**, 1247-1250.
236. Shimada, Y., Yonemura, S., Ohkura, H., Strutt, D., and Uemura, T. (2006) *Dev. Cell*, **10**, 209-222.
237. Nielsen, E., Severin, F., Backer, J. M., Hyman, A. A., and Zerial, M. (1999) *Nat. Cell Biol.*, **1**, 376-382.
238. Winter, C. G., Wang, B., Ballew, A., Royou, A., Karess, R., Axelrod, J. D., and Luo, L. (2001) *Cell*, **105**, 81-91.
239. Copp, A. J., and Greene, N. D. (2010) *J. Pathol.*, **220**, 217-230.
240. Kibar, Z., Torban, E., McDermid, J. R., Reynolds, A., Berghout, J., Mathieu, M., Kirillova, I., de Marco, P., Merello, E., Hayes, J. M., Wallingford, J. B., Drapeau, P., Capra, V., and Gros, P. (2007) *N. Engl. J. Med.*, **356**, 1432-1437.
241. Wang, Y. (2009) *Mol. Cancer Ther.*, **8**, 2103-2109.
242. Weeraratna, A. T., Jiang, Y., Hostetter, G., Rosenblatt, K., Duray, P., Bittner, M., and Trent, J. M. (2002) *Cancer Cell*, **1**, 279-288.
243. Kurayoshi, M., Oue, N., Yamamoto, H., Kishida, M., Inoue, A., Asahara, T., Yasui, W., and Kikuchi, A. (2006) *Cancer Res.*, **66**, 10439-10448.
244. Axelrod, J. D., Matsuno, K., Artavanis-Tsakonas, S., and Perrimon, N. (1996) *Science*, **271**, 1826-1832.
245. Munoz-Descalzo, S., Sanders, P. G., Montagne, C., Johnson, R. I., Balayo, T., and Arias, A. M. (2010) *Fly (Austin)*, **4**, 182-193.
246. Strutt, D., Johnson, R., Cooper, K., and Bray, S. (2002) *Curr. Biol.*, **12**, 813-824.
247. Das, G., Reynolds-Kenneally, J., and Mlodzik, M. (2002) *Dev. Cell*, **2**, 655-666.
248. Fanto, M., and Mlodzik, M. (1999) *Nature*, **397**, 523-526.
249. Cooper, M. T., and Bray, S. J. (1999) *Nature*, **397**, 526-530.
250. Robinson, D. R., Wu, Y. M., and Lin, S. F. (2000) *Oncogene*, **19**, 5548-5557.
251. Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., et al. (1984) *Nature*, **309**, 418-425.
252. Hafen, E., Basler, K., Edstroem, J. E., and Rubin, G. M. (1987) *Science*, **236**, 55-63.
253. Benzer, S. (1967) *Proc. Natl. Acad. Sci. USA*, **58**, 1112-1119.
254. Harris, W. A., Stark, W. S., and Walker, J. A. (1976) *J. Physiol.*, **256**, 415-439.
255. Tomlinson, A., and Ready, D. F. (1986) *Science*, **231**, 400-402.
256. Reinke, R., and Zipursky, S. L. (1988) *Cell*, **55**, 321-330.
257. Basler, K., and Hafen, E. (1988) *Cell*, **54**, 299-311.
258. Simon, M. A., Bowtell, D. D., and Rubin, G. M. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 8333-8337.
259. Rogge, R. D., Karlovich, C. A., and Banerjee, U. (1991) *Cell*, **64**, 39-48.
260. Simon, M. A., Bowtell, D. D., Dodson, G. S., Laverty, T. R., and Rubin, G. M. (1991) *Cell*, **67**, 701-716.
261. Bonfini, L., Karlovich, C. A., Dasgupta, C., and Banerjee, U. (1992) *Science*, **255**, 603-606.
262. Fortini, M. E., Simon, M. A., and Rubin, G. M. (1992) *Nature*, **355**, 559-561.
263. Simon, M. A., Dodson, G. S., and Rubin, G. M. (1993) *Cell*, **73**, 169-177.
264. Olivier, J. P., Raabe, T., Henkemeyer, M., Dickson, B., Mbamalu, G., Margolis, B., Schlessinger, J., Hafen, E., and Pawson, T. (1993) *Cell*, **73**, 179-191.
265. Gaul, U., Mardon, G., and Rubin, G. M. (1992) *Cell*, **68**, 1007-1019.
266. Dickson, B., Sprenger, F., Morrison, D., and Hafen, E. (1992) *Nature*, **60**, 600-603.
267. Lu, X., Melnick, M. B., Hsu, J. C., and Perrimon, N. (1994) *EMBO J.*, **13**, 2592-2599.
268. Brunner, D., Oellers, N., Szabad, J., Biggs, W. H., 3rd, Zipursky, S. L., and Hafen, E. (1994) *Cell*, **76**, 875-888.
269. Bohmann, D., Ellis, M. C., Staszewski, L. M., and Mlodzik, M. (1994) *Cell*, **78**, 973-986.
270. Brunner, D., Ducker, K., Oellers, N., Hafen, E., Scholz, H., and Klambt, C. (1994) *Nature*, **370**, 386-389.
271. Kauffmann, R. C., Li, S., Gallagher, P. A., Zhang, J., and Carthew, R. W. (1996) *Genes Dev.*, **10**, 2167-2178.
272. Raabe, T. (2000) *Biochim. Biophys. Acta*, **1496**, 151-163.
273. Blume-Jensen, P., and Hunter, T. (2001) *Nature*, **411**, 355-365.
274. Gschwind, A., Fischer, O. M., and Ullrich, A. (2004) *Nat. Rev. Cancer*, **4**, 361-370.
275. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. (1987) *Science*, **235**, 177-182.
276. Humphrey, P. A., Wong, A. J., Vogelstein, B., Zalutsky, M. R., Fuller, G. N., Archer, G. E., Friedman, H. S., Kwatra, M. M., Bigner, S. H., and Bigner, D. D. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 4207-4211.
277. Noble, M. E., Endicott, J. A., and Johnson, L. N. (2004) *Science*, **303**, 1800-1805.
278. Capdeville, R., Buchdunger, E., Zimmermann, J., and Matter, A. (2002) *Nat. Rev. Drug Discov.*, **1**, 493-502.
279. Reck, M., and Gatzemeier, U. (2005) *Respir. Med.*, **99**, 298-307.
280. Bukowski, R. M., Yasothan, U., and Kirkpatrick, P. (2010) *Nat. Rev. Drug Discov.*, **9**, 17-18.
281. Moy, B., Kirkpatrick, P., Kar, S., and Goss, P. (2007) *Nat. Rev. Drug Discov.*, **6**, 431-432.
282. Freeman, M. (1996) *Cell*, **87**, 651-660.
283. Dominguez, M., Wasserman, J. D., and Freeman, M. (1998) *Curr. Biol.*, **8**, 1039-1048.
284. Schweitzer, R., Howes, R., Smith, R., Shilo, B. Z., and Freeman, M. (1995) *Nature*, **376**, 699-702.
285. Golembo, M., Schweitzer, R., Freeman, M., and Shilo, B. Z. (1996) *Development*, **122**, 223-230.
286. Freeman, M. (1994) *Mech. Dev.*, **48**, 25-33.
287. Miller, D. T., and Cagan, R. L. (1998) *Development*, **125**, 2327-2335.
288. Golic, K. G., and Lindquist, S. (1989) *Cell*, **59**, 499-509.
289. Xu, T., and Rubin, G. M. (1993) *Development*, **117**, 1223-1237.
290. Wehrli, M., Dougan, S. T., Caldwell, K., O'Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Schejter, E., Tomlinson, A., and DiNardo, S. (2000) *Nature*, **407**, 527-530.
291. Newsome, T. P., Asling, B., and Dickson, B. J. (2000) *Development*, **127**, 851-860.

292. Rubin, G. M., and Spradling, A. C. (1982) *Science*, **218**, 348-353.
293. Groth, A. C., Fish, M., Nusse, R., and Calos, M. P. (2004) *Genetics*, **166**, 1775-1782.
294. Bischof, J., Maeda, R. K., Hediger, M., Karch, F., and Basler, K. (2007) *Proc. Natl. Acad. Sci. USA*, **104**, 3312-3317.
295. Oberstein, A., Pare, A., Kaplan, L., and Small, S. (2005) *Nat. Methods*, **2**, 583-585.
296. Horn, C., and Handler, A. M. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 12483-12488.
297. Brand, A. H., and Perrimon, N. (1993) *Development*, **118**, 401-415.
298. Bonini, N. M., Bui, Q. T., Gray-Board, G. L., and Warrick, J. M. (1997) *Development*, **124**, 4819-4826.
299. Basler, K., Yen, D., Tomlinson, A., and Hafen, E. (1990) *Genes Dev.*, **4**, 728-739.
300. Crew, J. R., Batterham, P., and Pollock, J. A. (1997) *Dev. Genes Evol.*, **206**, 481-493.
301. Wang, T., Jiao, Y., and Montell, C. (2007) *J. Cell Biol.*, **177**, 305-316.
302. Yoshihara, Y., Mizuno, T., Nakahira, M., Kawasaki, M., Watanabe, Y., Kagamiyama, H., Jishage, K. I., Ueda, O., Suzuki, H., Tabuchi, K., Sawamoto, K., Okano, H., Noda, T., and Mori, K. (1999) *Neuron*, **22**, 33-41.
303. Dietzl, G., Chen, D., Schnorrer, F., Su, K. C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblaue, S., Couto, A., Marra, V., Keleman, K., and Dickson, B. J. (2007) *Nature*, **448**, 151-156.
304. Roberson, E. D., and Mucke, L. (2006) *Science*, **314**, 781-784.
305. Greeve, I., Kretschmar, D., Tschape, J. A., Beyn, A., Brellinger, C., Schweizer, M., Nitsch, R. M., and Reifegerste, R. (2004) *J. Neurosci.*, **24**, 3899-3906.
306. Finelli, A., Kelkar, A., Song, H. J., Yang, H., and Konsolaki, M. (2004) *Mol. Cell. Neurosci.*, **26**, 365-375.
307. Crowther, D. C., Kinghorn, K. J., Miranda, E., Page, R., Curry, J. A., Duthie, F. A., Gubb, D. C., and Lomas, D. A. (2005) *Neuroscience*, **132**, 123-135.
308. Dawson, T. M., and Dawson, V. L. (2003) *Science*, **302**, 819-822.
309. Feany, M. B., and Bender, W. W. (2000) *Nature*, **404**, 394-398.
310. Takahashi, M., Kanuka, H., Fujiwara, H., Koyama, A., Hasegawa, M., Miura, M., and Iwatsubo, T. (2003) *Neurosci. Lett.*, **336**, 155-158.
311. Chen, L., and Feany, M. B. (2005) *Nat. Neurosci.*, **8**, 657-663.
312. Bates, G. P. (2005) *Nat. Rev. Genet.*, **6**, 766-773.
313. Jackson, G. R., Salecker, I., Dong, X., Yao, X., Arnheim, N., Faber, P. W., MacDonald, M. E., and Zipursky, S. L. (1998) *Neuron*, **21**, 633-642.
314. Kazemi-Esfarjani, P., and Benzer, S. (2000) *Science*, **287**, 1837-1840.
315. Warrick, J. M., Chan, H. Y., Gray-Board, G. L., Chai, Y., Paulson, H. L., and Bonini, N. M. (1999) *Nat. Genet.*, **23**, 425-428.