1 The Introspective Frog

In 1992 a high school student in Ontario, Canada, found a toad in her yard. Deidre knew something was wrong when it didn't open its eyes as she picked it up. When she looked inside its mouth, she was shocked to see a pair of eyes looking back at her (Fig. 1.1). At first, she thought the eyes might belong to another toad that it had swallowed, but she soon realized that they were attached to the roof of its mouth. She took the toad inside and tried feeding it some worms, but it would only eat them if she placed them directly into its mouth [142].

Deidre contacted the local newspaper, and they sent a crew to her home to see the frog for themselves. When the staff photographer, Scott Gardner, got the call over his two-way radio, he rolled his eyes in disbelief, suspecting that the dispatcher was just playing a prank on him, but upon his arrival he saw that the introspective

Fig. 1.1. A frog whose eyes developed inside its mouth. The toad (*Bufo americanus*) reacted to motions only when it gaped [276]. The only known similar case was a leopard frog (*Lithobates pipiens*) found in Minnesota in 1996, with one normal external eye and one internal eye that hung down from the roof of the mouth on a stalk of flesh [1183]. Photo (used with permission) by Scott Gardner, staff photographer for *The Hamilton Spectator*.

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amphibian was quite real after all [402]. By this time Deidre had named it Gollum after the semi-aquatic creature in Tolkien's *Lord of the Rings*. The crew got him to open his mouth by tapping his lips with some tasty insects.

The next day Deidre took Gollum to a herpetologist at the University of Guelph. Professor Bogart had seen a lot of amphibian abnormalities over the years [524], but never one like this. Like a doctor at an emergency clinic, he recorded the essential facts of the patient's presentation [1268]: "male, *Bufo americanus* (common in Ontario), two inches long, and at least two years old." He was surprised that a nearly blind toad had survived in the wild for so long.

As for how Gollum got this way in the first place, Dr. Bogart could not be sure. He surmised that the eyes had developed upside-down, but he could not tell whether the cause was genetic or environmental. He wanted to mate Gollum to see whether the trait was heritable, but Deidre adamantly refused to loan her pet to him for that purpose. Indeed, she even declined to donate Gollum's body for an autopsy after he died. Only one other similar frog was subsequently found in Minnesota [1183], which makes an external agent (e.g., a pesticide) less likely.

The aquatic habitat of tadpoles exposes them to potential damage by parasites and predators [653], though the bilateral symmetry of Gollum's eye trait would seem to argue against any such targeted external injury. Unlike other vertebrates, frogs have muscles that can depress the eyes toward the oral cavity to aid in swallowing, but Gollum's phenotype cannot be explained by eye rotation alone because the skin atop the head was unbroken where his eyelids should have been, and there was no sheath of palatal skin covering the lenses of his eyes.

In theory, Gollum's palatal skin could have been somehow pierced by fully formed eyes, but a more plausible explanation, based on what we know about vertebrate eye development (see below), is that his palatal skin was incorporated into the eyes themselves when his retinas accidentally grew down toward his mouth.

GP-1: Inductive signaling can enhance precision

Nobel laureate Sydney Brenner (1927–2019) was famous for his wit [759], and one of his cleverest sayings was that embryonic cells acquire their fates based either on who their parents were – the "British Plan" – or on who they happen to know – the "American Plan" [1060]. Or, to paraphrase, embryonic cells adopt distinct roles based on (1) instructions they inherit via cell lineage or (2) signals they receive from their neighbors. The nematode embryos studied by Brenner's lab primarily use the first kind of source [1207], whereas vertebrate embryos routinely rely on the second one.

Proof of intercellular signaling during vertebrate eye development was adduced by Hans Spemann (1869–1941), the first embryologist to win a Nobel Prize. Spemann recounted his experiments in the 1938 book *Embryonic Development and Induction* [1187]. His key conclusion was that the optic cup induces the lens – the first kind of induction ever documented [1100]. The optic cup grows out from the

developing brain (neural tube) to form the retina, and the lens arises wherever the cup contacts the overlying ectoderm (prospective epidermis). In some frog species a lens can be elicited virtually anywhere in the head or trunk region by transplanting an optic cup (or antecedent vesicle) beneath the ectoderm [974], thus implying a causal relationship between cup and lens (boldface added):

> The nature of these potencies [of skin regions outside the normal site of lens formation] especially can be ascertained only if, as I suggested, the optic cup is brought into contact with foreign parts of the epidermis, either by transplantation of the optic cup itself or of the epidermis covering it ... The most incontestable results are obtained by the first-mentioned method, in which the optic vesicle is exposed, cut off, and pushed backward under the [trunk] epidermis. This experiment was first made by W. H. Lewis ... on *Rana sylvatica* and *palustris*, with the result that, in numerous cases, **a lens formed above the transplanted optic vesicle**. After a short development the lens was still in connection with the skin and thus indicated its [trunk skin] origin. [1187]

It is therefore possible that Gollum's optic cups took a wrong turn and wound up inducing lenses in the roof of his mouth (Fig. 1.2). Such a detour would explain why his eyes looked normal despite being displaced, as well as why they had no cloudy patina of palatal skin, as that skin would have become transparent lens tissue. One obvious way to test this hypothesis would be to see whether an artificially transplanted optic cup can induce a lens in the palatal ectoderm of this species. It is a shame that no offspring were obtained to see if Gollum's anomaly was genetic. The defect remains enigmatic, partly because we don't yet know what factors dictate the normal trajectories of the optic cups.

A priori one might have imagined that evolution could have evoked the vertebrate lens and retina from separate sites within the embryo and then fitted them together, but that would have run the risk of misalignment. As anyone who wears glasses realizes, clear vision requires fine precision, and induction of the lens by the optic cup guarantees fidelity of fit. If lens–retina coordination is so useful for acuity, then we should see it in the eyes of non-vertebrates as well. Indeed, lens and retina development are intertwined in the similar but non-homologous eyes of cephalopods [715].

Based on genetic studies in the mouse, the chief intercellular signals that induce the vertebrate lens are Bone Morphogenetic Proteins (BMPs) [500], with other signaling pathways playing supporting roles [262]. Recent research shows that ectodermal cells cannot respond to these inductive BMPs unless they are primed to do so in advance [647]. The main priming agent that makes them "competent" is the conserved transcription factor Pax6, though other regulatory proteins interact with Pax6 in a complicated genetic network [262].

GP-1 tangent: Bract induction

A much simpler instance of induction concerns the "bract" – a tiny cuticular structure in flies. The inducer in this case is a ligand of the Epidermal Growth Factor Receptor (EGFR) pathway, and the competence agent is Distal-less
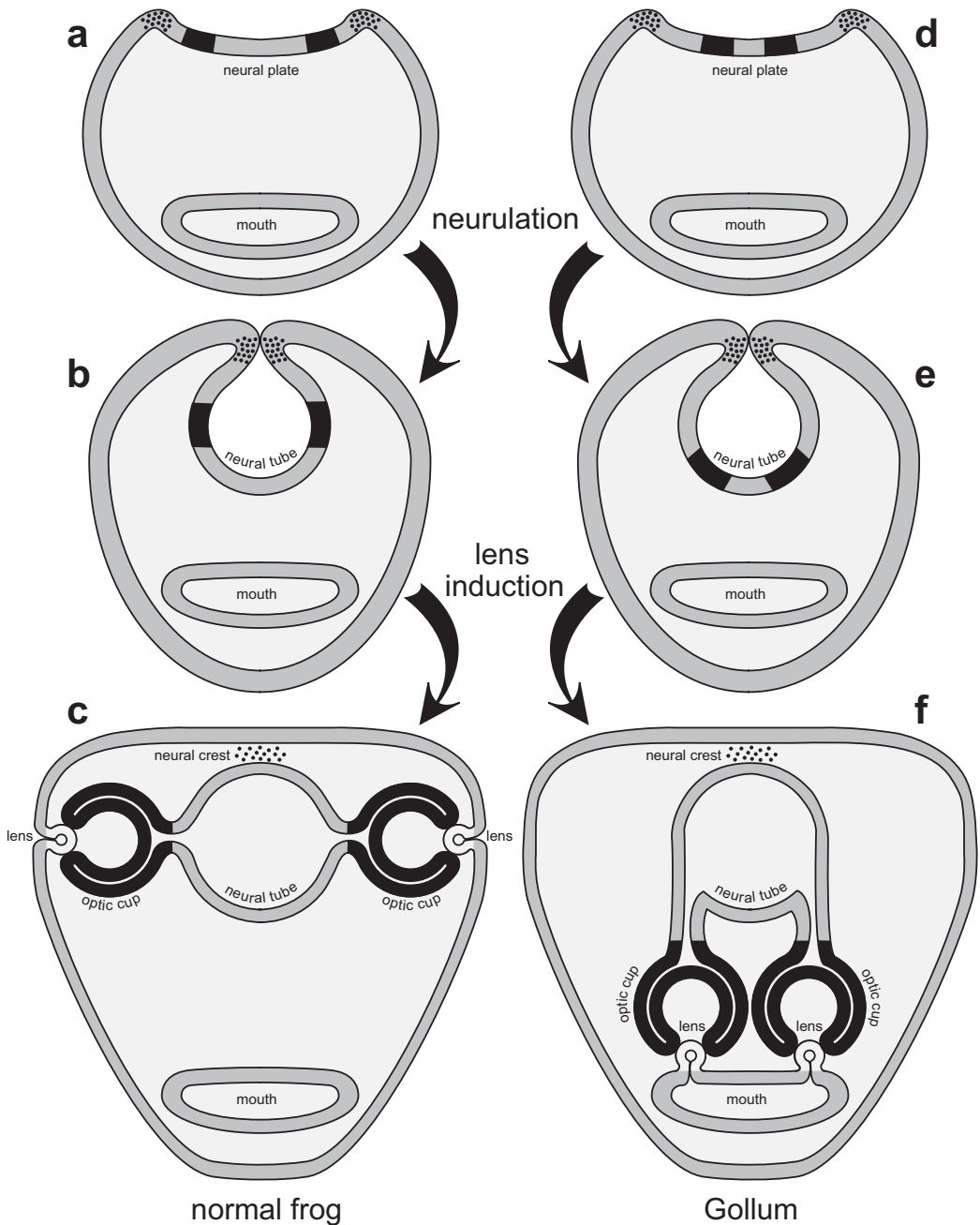
Fig. 1.2. Hypothetical etiology of Gollum's eyes. The development of a normal frog's eyes (**a**, **b**, **c**) is compared to that of Gollum's eyes (**d**, **e**, **f**), assuming that Gollum's optic cups suffered a ventral detour from the orthodox lateral trajectory. Stages are depicted schematically: **a**, **d**: early neurula; **b**, **e**: late neurula; and **c**, **f**: lens induction, showing invagination of the lens placode to form a vesicle. (A similar induction elicits the auditory vesicle that becomes our inner ear [742].) All panels are coronal cross-sections of the head

(Dll) – a protein, which, like Pax6, contains an ancient homeodomain (DNA-binding) motif. The rationale for delving into this vignette is to briefly show how induction can ensure precision (GP-1) on a *cellular* scale, not just at the tissue or organ levels as in the lens-induction case.

Bracts are thorn-like protrusions that are secreted by single cells in fruit flies. They never occur alone but are always found adjacent to bristles on the legs and wings where the gene *Distal-less* (*Dll*) is expressed, and indeed, loss-of-function (LOF) mutations in *Dll* block bract – but not bristle – development [174]. For many years circumstantial evidence continued to mount, arguing that bristles induce bracts, but definitive proof only came in 2002, when the inductive signal was identified as Spitz, a ligand of the EGFR pathway [289,556].

Mechanosensory bristles in *Drosophila melanogaster* are formed by a cluster of cells, all of which descend from a common ancestor called the "sensory organ precursor" (SOP). SOPs arise at consistent sites within the fly epidermis during metamorphosis (Fig. 1.3). They undergo three mitoses to yield five cells [420,1053], each of which acquires a unique identity via instructions that it inherits from the SOP, obeying Brenner's British Plan, where lineage dictates destiny.

In contrast, the bract cell is recruited into the bristle complex via the American Plan, where your fate depends on who you know. No one knew *which* member of the bristle clan induces the bystander until 2012, when the mystery was solved by Ying Peng and Jeff Axelrod. Their paper not only indicted the socket cell beyond any shadow of a doubt, but also uncovered a novel mode of close-range induction [1011]. Instead of "spitting" Spitz willy-nilly in its vicinity, which is the norm for paracrine inducers [1016], the socket cell reaches under the epidermis to tickle the unsuspecting neighbor with a Spitz-laden lamellipodium.

The revelation of this rude gesture on the part of the socket cell made perfect sense to aficionados of the bract world, because it neatly solved another nagging riddle. Why do bracts only develop on the proximal side of bristle sockets? Now we know. They do so because socket cells only extend their subterranean feelers in a proximal direction (toward the body). How do they determine which way is proximal (vs. distal)? They use the equivalent of a compass to tell which direction is which. It is called the Planar Cell Polarity (PCP) pathway – an evolutionarily ancient "app" that animal cells rely upon to navigate all sorts of challenges during development [561].

Fig. 1.2. (*cont.*)

(dorsal above), with ectoderm in gray. NB: Neurulation is induced by the underlying notochord (not shown) [236]. Most of the gut is endodermal [208], but the buccal cavity ("mouth") is colonized by ectoderm [205,1184]. Black zones in **a** and **d** are optic cup primordia, which might have been more medial in Gollum as a result of erroneous patterning. Cups in **c** and **f** are black, lenses are white, and neural crest cells are black dots. Lenses are larger relative to optic cups than shown here. For further details see Figure 1.5 and [262,882]. After [557,1187].
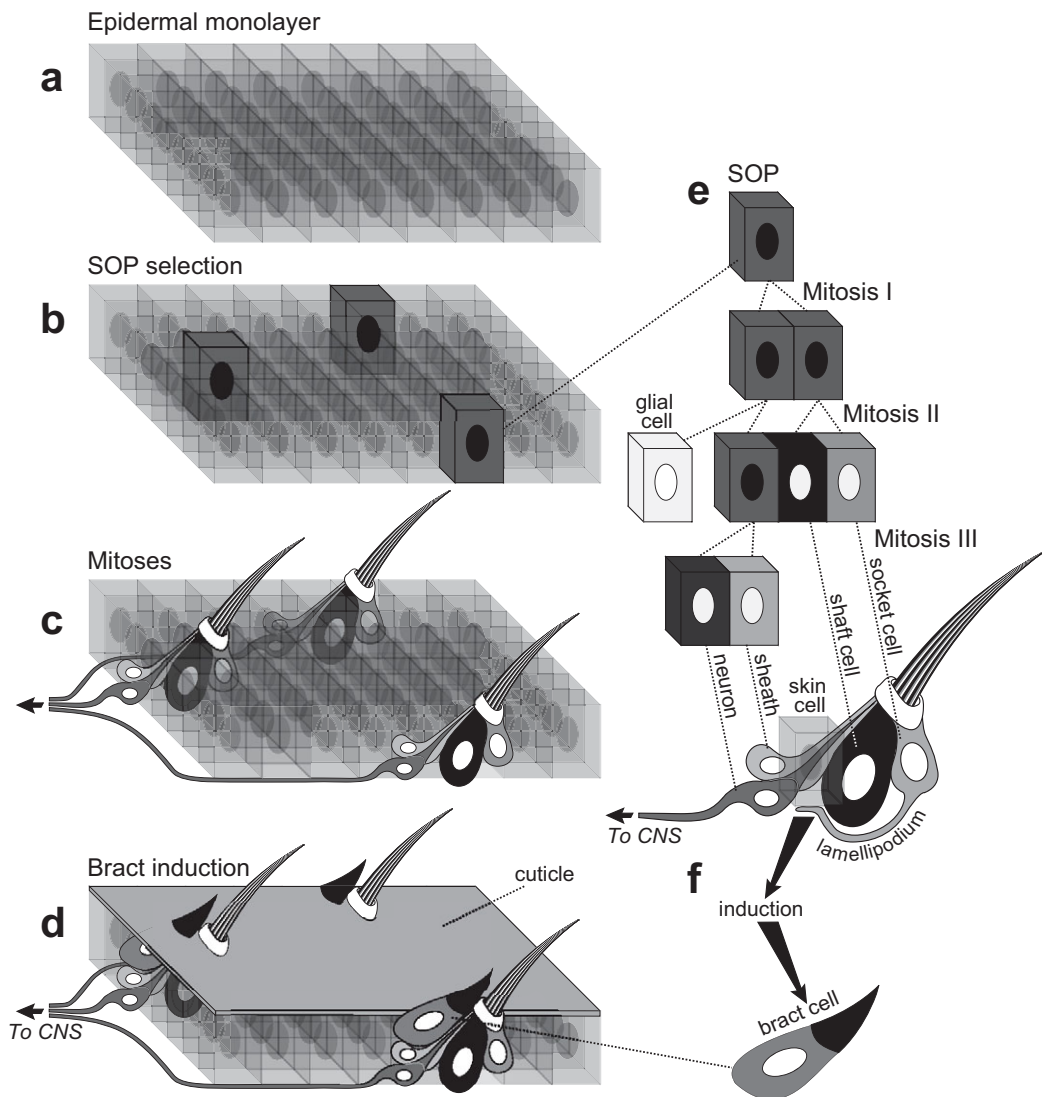
Fig. 1.3. Induction of bract cells during fly leg metamorphosis. In panels **a**–**d**, a region of fly leg epidermis is drawn schematically, showing the emergence of mechanosensory bristles from single sensory organ precursor (SOP) cells. In all panels, proximal is to the left and distal to the right. **a**. Cells are depicted as translucent boxes with a nucleus (oval) inside. Their actual packing, however, is not nearly so regular as shown in this array. **b**. Three SOPs (darker boxes with black nuclei) are drawn as examples. **c**. Completion of differentiative mitoses. **d**. Bract cell formation. **e**. Pedigree of the bristle lineage, with glial cell to the side because it will migrate away [420,1053]. **f**. Induction of a bract cell by the socket cell, which extends a lamellipodium proximally to reach the nearest neighboring (ordinary skin) cell. Induction actually occurs before terminal differentiation of the bristle. Axons of neurons coalesce into bundles as they leave the skin, headed for the central nervous system (CNS). The size of the bract cell is exaggerated. Redrawn with modification from [552,555].
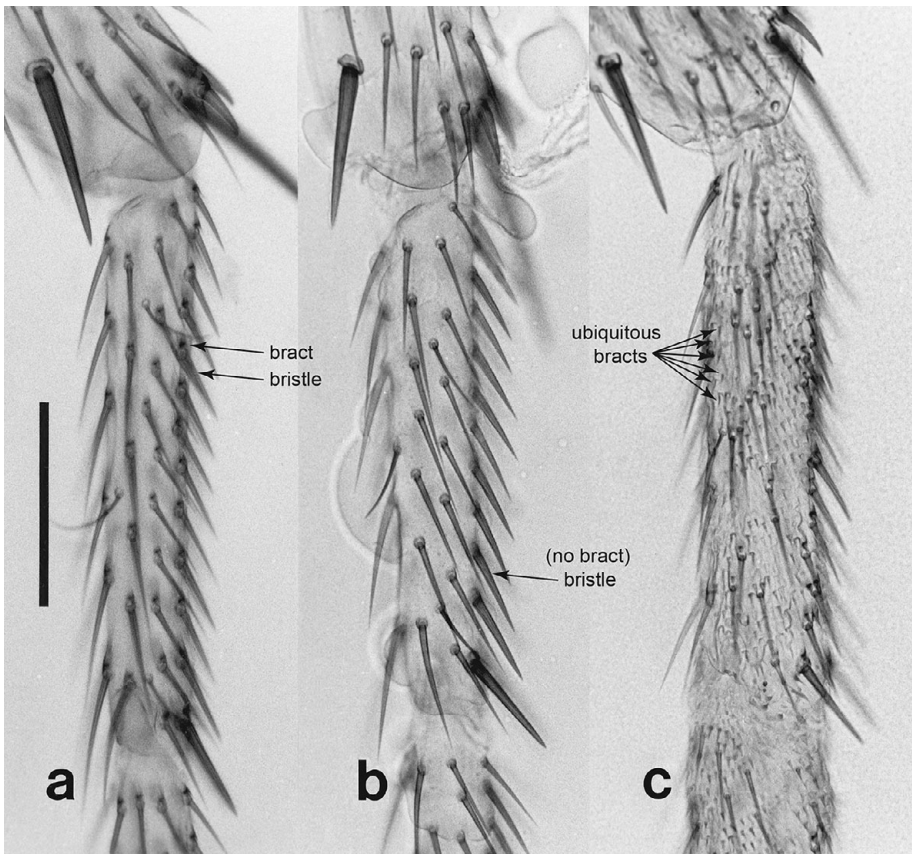
Fig. 1.4. Modulation of bract induction by manipulating the EGFR pathway. Each panel shows the anterior face of the basitarsal segment of a right *D. melanogaster* second leg (proximal at top; scale bar = 100 μm). **a**. Normal phenotype. Bracts are the tiny triangular structures above most bristle sockets. **b**. Fly whose EGFR protein was inactivated (total LOF). No bracts develop. A similar bractless phenotype is seen for *Distal-less*-LOF [174]. **c**. Fly whose EGFR pathway was hyperactivated (extreme GOF) by overexpressing the *Ras1* gene. Most of the skin cells have formed thin bracts at the expense of bristles. See [289] for a similar analysis that yielded comparable phenotypes. From [556]; used with permission from *Elsevier/RightsLink*.

It is possible to override this directional preference by artificially driving the EGFR pathway to higher levels within the entire bristle complex or within the epidermis as a whole, which results in many more bracts than normal being elicited. An example of such a hypermorphic phenotype is shown in Figure 1.4c.

As tidy as this tale might seem, there are still loose ends that remain to be tied up. Chief among them is the function of the bract itself. It lacks any connection to a nerve, nor does it restrict the motion of the bristle shaft, so it can't be acting in touch sensation. Indeed, its removal by mutation does not appear to leave the fly worse off than before. Such silly trifles (think of the muscles that wiggle your ears [557]) are

often explicable as vestiges of antecedent devices that did serve a purpose in an ancient ancestor, but no such explanation can rescue us here. Some such baubles help animals entice mates (e.g., the gaudy fan of the peacock), but bracts are not sexually dimorphic, nor does fly vision seem good enough to discern bracts from a distance anyway.

Hence, we are left with the puzzle of why evolution has gone to the trouble of deploying an intricate inductive mechanism to situate a seemingly needless structure (the bract) next to a functional one (the bristle). Conceivably, the answer lies buried in the fly genome somewhere, but there are likely to be few Don Quixotes willing to devote much time trying to find it. Nevertheless, the military precision with which this induction is executed illustrates the power of intercellular signals to build multicellular ensembles. The epitome of architectural accuracy is the ommatidium of the insect compound eye [201], where an elaborate cascade of sequential inductions assembles the cell types within each modular conglomerate [57,736].

GP-2: Embryos tend to build anatomy by origami

Animals are three-dimensional organisms, but many of their organs begin as two-dimensional sheets that fold extensively to attain their final shapes [436,1311,1428]. This 2D → 3D "origami" strategy [377,658] is obvious in Figure 1.2, where (1) the central nervous system emerges by rolling the surface into a tube [225], (2) the optic cups originate by outgrowth from the walls of that tube [84,183], and (3) the lenses arise by inpocketing of the surface wherever the cups encounter the ectoderm on their outward journey [262]. All three of these examples involve ectoderm, but the endoderm undergoes a comparable contortion called "gastrulation" to form the digestive tract [1181].

A brief aside on terminology may be useful here. Ectoderm, mesoderm, and endoderm are the primary germ layers (outer, middle, and inner) of animal embryos as defined at the gastrula stage, and each has its own talents and limitations [506]. Mesoderm employs a mesenchymal type of tissue plan (loose 3D network) more often than an epithelial one (2D sheets), though somites are a blatant exception [667], as is our heart [242]. Then there is the neural crest, which behaves, *sui generis*, as a fourth germ layer. It starts within an ectodermal sheet but dissolves into a mesenchyme when the neural tube involutes (Fig. 1.2b,c) [668,1276], and the cells that are thus liberated from their epithelial bonds migrate all over the embryo to adopt various fates [156] but remain mostly mesenchymal [859].

Gastrulation and neurulation are such integral aspects of development that we take them for granted, yet there is no obvious reason for animals to have settled upon the 2D gimmickry of origami versus other ways of making tubes [1237], such as the excavation of solid 3D cylinders [29,53] (that is how neurulation proceeds in actinopterygians [3]). Presumably, evolution is to blame. The first animals are thought to have had their digestive and nervous tissues on their surfaces [595],

while their descendants at some point tucked these tissues inside, probably for protection [506,1114].

The 2D maneuvers we witness at the tissue level must be driven by shape changes at the cellular level [293,604,659], and ultimately by cytoskeletal motors at the molecular level [846,853]. Conveniently, we can travel down into that mechanized underworld by following the next steps in the story of lens development [633], which begins with lens induction (Fig. 1.2c) [202]. Along the way we will encounter many evolutionarily conserved gadgets that govern the development of a wide variety of other organs across the entire animal spectrum.

The first overt sign of lens formation is a thickening of the ectoderm into a placode that then invaginates. Indeed, virtually all epithelial invaginations follow this same recipe, with cells changing from a cuboidal to a columnar shape as a prerequisite for involution [1006]. Why must cells be primed in this geometrical way? The answer might be that the longer intercellular junctions of tall (vs. short) cells can serve as flexible hinges [1123], or that the greater stiffness of the cytoskeleton gives them added leverage [1006], or both.

After the placode cells thicken, these cylindrical cells constrict their apices to adopt a conical shape [271], which causes the placode to buckle into a pit (Fig. 1.5a) [724]. The pit's concavity is adjusted by a contrivance that is nearly universal in the animal kingdom: the actin-remodeling GTPases RhoA and Rac1 act as "tuning knobs" to increase or decrease the bowl's curvature to whatever "Goldilocks" setting the genome happens to specify [203].

This dimpled pit then detaches from the overlying ectoderm to form a vesicle (Fig. 1.5b), but the vesicle cannot remain hollow if it is to focus images as accurately as the glass lens of a camera [75]. To accomplish that, it must become a solid ball of cytoplasm so that its refractive index is homogenized. That final state could theoretically be achieved in a multitude of ways, but vertebrates tend to follow the same procedure [262]. The protocol entails filling the vesicle cavity with cells that elongate from the side of the wall that is closest to the future retina (Fig. 1.5b,c) [44].

Are the cells there *intrinsically* prone to elongate? No. They are induced to do so by an *external* signal (Fgf) that diffuses toward the lens from the optic cup [860]. The existence of such a signal was inferred in 1963 when researchers excised a lens from a chicken embryo and returned it upside-down. If this experiment was performed just before the stage depicted in Figure 1.5b, then the cells that had been stretching came to an abrupt halt, and the cells on the opposite side (now facing the future retina) began lengthening instead [243].

Regardless of their source, the elongating cells eventually fill the cavity to create the "primary" lens – a solid ball topped by a remnant layer of cuboidal cells (Fig. 1.5c). Theoretically, this lens should be able to grow as the newborn animal gets bigger, but it cannot do so because its cells have forfeited the ability to undergo mitosis: their nuclei disintegrate during differentiation to enhance acuity even more since DNA and cytoplasm differ in refractive index [76]. Given the clear benefit of this tactic, it is not surprising that cephalopod lens cells also lose their nuclei [199],
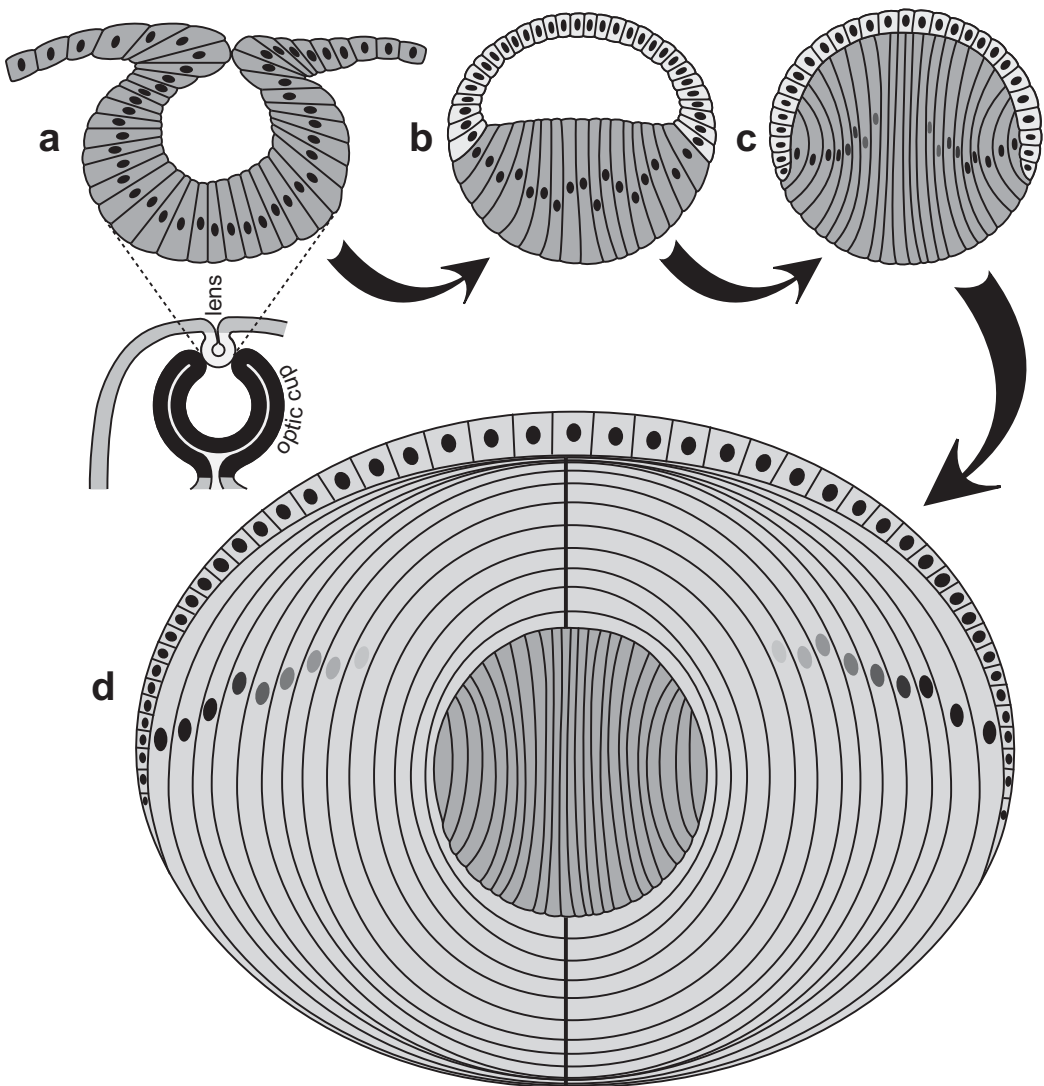
Fig. 1.5. Maturation of the lens after induction. A mouse lens is cross-sectioned at four stages of development (rotated 90° from Fig. 1.2c). **a**. Lens pit, whose cells constrict apically in response to BMP [646]. **b**. Lens vesicle, whose cavity is being filled with cells that cease mitosis and elongate (dark gray) [44], while remaining cells (light gray) continue to divide [227,794]. **c**. Primary lens, whose cell nuclei (ovals) disintegrate (black → gray) from the middle outward. **d**. Secondary lens (light gray), which encircles the primary lens (dark gray). Mitoses push cells toward the equator, where they stretch around the primary lens to meet a counterpart at the suture [261]. Nuclei vanish (as in **c**), which ensures transparency [76]. NB: The ectodermal surface layer (future cornea) left over after the vesicle detaches is omitted from **b**–**d**. The Pax6-dependent process whereby the crests in **a** merge to liberate the lens malfunctions in Peters' anomaly – a rare disease where a connecting stalk persists [261]. The lens (inset in **a**) is much larger relative to the optic cup than depicted here. The zebrafish lens arises by an alternative (3D → 3D) pathway [487], and lens regeneration entails budding

and insect lenses are made of extracellular cuticle where the issue of nuclear sacrifice is moot [1195].

Vertebrates solve the problem of restricted proliferation in a peculiar way (Fig. 1.5d) [262]. Cuboidal cells continue to divide, and when daughter cells get pushed to the equator, they increase in length 100- to 1000-fold to encircle the primary lens from both sides [1145]. Hence, an onion-like "secondary" lens comes to surround the primary lens, letting the overall lens retain a spheroidal shape as it grows [860]. Nuclei vanish, like those of the primary lens, but before they do so, both cell types purify their cytoplasm by reducing it to a single type of protein called a crystallin. Cephalopods do this too but use non-homologous proteins [546]. In order to avoid spherical aberration, vertebrates and cephalopods both vary the concentration of crystallin proteins (and hence refractive index) as a function of radial distance from the lens center [75,171].

GP-2 tangent: Imaginal discs

The epitome of origami is embodied by the imaginal discs of flies and other insects that undergo total metamorphosis [379]. As an added bonus, the cellular mechanics of their epithelial acrobatics are well understood [659]. Imaginal discs get their name from the fact that they form parts of the imago (the Latin term for an adult insect) and from their resemblance to collapsed balloons (i.e., round and flat). Their key properties are nicely demonstrated by the leg disc (Fig. 1.6).

Each leg of the fruit fly begins as a 2D cluster of 10–20 ectodermal cells that are set aside from the surrounding surface cells, which form the larval skin [78]. The cluster withdraws into the body cavity during the embryonic period [830] and grows inside the larva until metamorphosis [222]. Initially it is a solid mass, but it delaminates to yield a hollow sac [830]. As it grows, the thicker (columnar) side of the sac acquires concentric folds [861] like a Danish pastry [1339]. These folds telescope out as the disc everts to create a hollow cylinder that becomes the adult leg.

Eversion of the leg disc takes a few hours [1272], but it can be sped up to just a few minutes by exposing discs to the enzyme trypsin [228]. Apparently, the columnar cells are spring-loaded to unleash their coils at the slightest provocation, like a Jack-in-the-box toy. Trypsin's power to trigger this release has been traced to its ability to simulate endogenous proteases [300]. Those proteases degrade extracellular-matrix (ECM) proteins (e.g., collagen) that restrain columnar-to-cuboidal transitions.

Fig. 1.5. (*cont.*)

from the iris or cornea [1328]! (Lens regeneration may have evolved in response to lens-eating parasites [575,974].) Curiously, the fly lens shares a number of features with the vertebrate lens [119,201]. See [89,1144] for geometrical perspective and [261] for unsolved puzzles. The gold medal for weirdest origami stunt must go to the hollow, spherical embryos of the colonial alga *Volvox*, which turn themselves inside out [856], and an analogous inversion occurs in a colonial choanoflagellate [161]. Redrawn from [262]; used with permission.
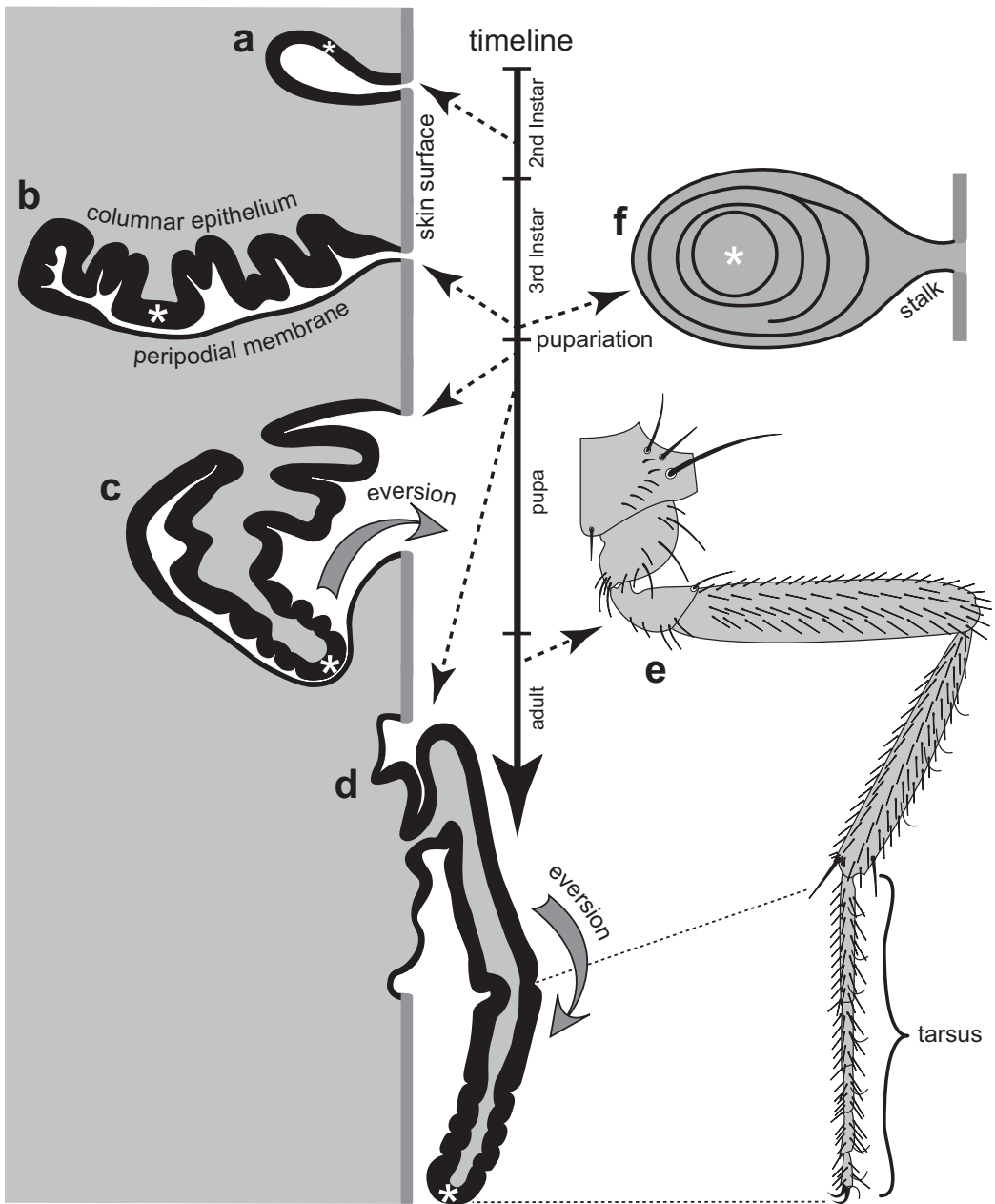
Fig. 1.6. Development of a second-leg disc in *D. melanogaster*. Black shapes (**a–d**) are cross-sections at successive stages (see timeline), with an asterisk marking the future tip of the leg. Cell boundaries and nuclei are omitted, as are the basement membrane, adepithelial cells, outgoing nerve, and overlying cuticle [555]. **a.** Mid-2nd instar, when the solid disc acquires a lumen to become a hollow sac [45,830,865]. All cells remain cuboidal until early 3rd instar, when one side thickens into a columnar epithelium, and the other side flattens into a

The columnar state itself is enforced by Dpp and Wingless – the two main signaling agents that govern the disc coordinate system [1384,1385].

How does the nascent leg elongate without widening to an equal extent, as should occur by cell flattening alone? "Convergent-extension" couplings are common in animal embryos [1148], and they typically involve cell intercalation [1149]. It turns out that choreographed cell rearrangements are also instrumental in both leg [381,1272] and wing [300,659] eversion. In both cases the anisotropy stems from a polarized localization of Myosin-II, but amazingly, none of the known PCP pathways [570] directs this localization [300].

Aside from ECM degradation, anisotropic cell flattening, and cell rearrangement, leg disc eversion is driven, at least in part, by a systemic increase in blood pressure [376], which blows the leg up like a balloon [1272]. Anyone who has ever watched an insect cringe after emerging from its pupal case knows how critical this gimmick is for straightening the wing blades. As the imago contracts its body wall musculature, its hemolymph gets pumped along the wing veins (see Fig. 3.5), which act as rigid struts until the cuticle hardens and the insect can finally relax [999].

Beetles are interesting in this regard because they use hydraulics not only to flatten their wings, but also to inflate their nasal horns (if they have them) [855] and to deploy their hindwings for takeoff throughout their adult lives [1234].

Ancestral insects had two pairs of wings (like dragonflies), but only one pair is needed for flight, so evolution could re-purpose one pair any way it saw fit, so to speak. The dipteran order of flies turned the hindwings into gyroscope-like halteres (see Fig. 5.2) [560], while the coleopteran order of beetles turned the forewings into chitin-fortified elytra that protect the fragile hindwings (Fig. 1.7) [1295]. Most of us have seen a ladybird beetle shut its elytra upon landing, but we may not have noticed the gossamer hindwings disappearing beneath them as they are carefully tucked away.

A fuller understanding of this wing retraction "app" had to wait until 2017 when researchers cleverly replaced one elytron with a transparent acrylic replica so that they could observe the entire origami ritual [1101]. The gadgetry involved is too intricate to describe here since it would distract us along yet another tangent.

Fig. 1.6. (*cont.*)

squamous "peripodial membrane" [861] (cf. neural vs. pigment layers of the optic cup [913]). Both remain monolayers. **b.** Late 3rd instar. The columnar side has concentric folds (top view in **f**) [228] and remains attached to the surface by a thin stalk. **c, d.** Early pupal period. Folds telescope out, and the leg everts through the dilating stalk [1000] to form a hollow cylinder that secretes the adult cuticle. Ritualized contortions during eclosion from the pupal case (not shown) are reminiscent of the escape artist Harry Houdini [376]. **e.** Adult leg (anterior view). Creases in the pupal tarsus ultimately make the adult tarsal joints [426,485]. See [380] for details, [1229] for how folds arise, and [384,539] for how discs fuse at the midline. (First-leg discs share a membrane [890].) See [298,300,659] for wing disc eversion, which is driven not only by cell shuffling, cell flattening, and hydraulic pressure, but also by cell division, cell extrusion, shear forces, and cuticle stresses. Adapted from [228,381,555,1028,1272].
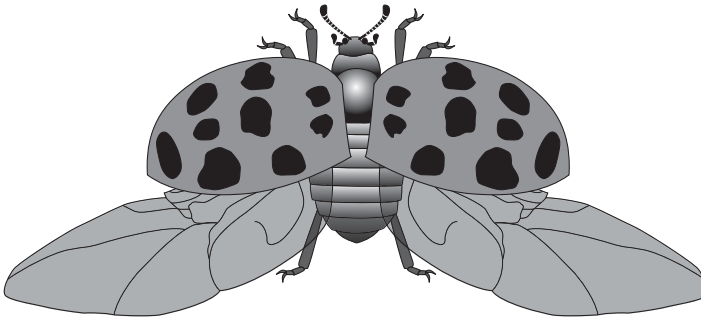
Fig. 1.7. Ladybird beetle. The delicate hindwings of ladybird beetles (e.g., the *Harmonia axyridis* drawn here) are deployed during flight, but when the insect lands, an elaborate folding ritual tucks them under decorative covers called elytra. Those elytra, which evolved from forewings, are made of heavily sclerotized cuticle. From [560].

Suffice it to say that the wings are ratcheted by sandwiching them between the upper surface of the squirming abdomen and the lower surface of the overlying elytra, and that bending is enabled by elastic hinges embedded in the vein lacework. Variations on this theme have been documented in earwigs [303], rove beetles [1102], shield bugs [103], and bamboo weevils [796].

By the time that beetles can fly, their wing cells have long since died – having fulfilled their task of secreting the adult cuticle. The elastic and rigid areas within that cuticle dictate how the wing will passively bend under external forces. In contrast, the origami of embryonic epithelia involves active bending in response to internal forces. Aside from the developmental case studies discussed above, other remarkable examples of 2D morphogenesis include our heart [242], brain [1250,1299], intestine [944], pancreas [247], ear [24], thyroid [208], and thymus [208].