CHAPTER ONE

Cell Lineage vs. Intercellular Signaling

Imaginal discs are hollow sacs of cells that make adult structures during metamorphosis. They are so named because "imago" is the old term for an adult insect [4008], and their shape is discoid (i.e., flat and round like a deflated balloon) [377]. They arise as pockets in the embryonic ectoderm and grow inside the body cavity until the larva becomes a pupa, at which point they turn inside out ("evaginate") to form the body wall and appendages [3165]. In a *D. melanogaster* larva there are 19 discs (Fig. 1.1). Nine pairs form the head and thorax, and a medial disc forms the genitalia. The abdominal epidermis comes from separate cell clusters called "histoblast nests" [2301, 2648, 3647]. Unlike discs, histoblast nests remain superficial during larval life [927] and do not grow until the pupal stage [2650].

Given the diversity of cell types in the adult skin (e.g., bristles, sensilla, photoreceptors) and the commonality of their descent from one progenitor (the fertilized egg), it is natural to ask how cells specialize to adopt divergent roles. In principle, cells can acquire instructions from ancestors or contemporaries [1654]. More specifically, a cell can inherit predispositions from its mother ("intrinsic" mode), take cues from neighbors ("extrinsic" mode), or both [477, 1614, 2019, 2451, 3741]. The predispositions could be gene states, while the cues could be diffusible ligands [1144, 3182].

To the extent that fates are assigned intrinsically, there should be a rigid correspondence between (1) parts of the anatomy and (2) branches of the lineage tree [1362, 4086, 4087]. That is, a clone of cells descended from ancestral cell "x" should make structure "X", while another clone descended from ancestor cell "y" should make structure "Y". Moreover, these rules should be obeyed in every member of the species. *C. elegans* worms adhere closely to this strategy [1284, 4201, 4202], but flies do not [1839, 1881]. In *D. melanogaster*, the only adult structures that use an intrinsic mode are tiny sense organs [532, 1410, 3441]. All larger parts of the body use extrinsic mechanisms. Thus, the problem of how discs develop can be reduced to questions about how cells communicate [695]. Who signals to whom? Over what distance? With what molecules? To what end?

Discs are not clones

Proof that cell pedigrees are irrelevant for disc patterning was first provided in a 1929 paper [4180] by Alfred Henry Sturtevant (1891–1970) – a wunderkind of the Morgan lab [257, 2504, 2615]. Sturtevant studied a strain that produced freakish flies called "gynandromorphs" [2950]. Each such fly is a patchwork of purely male and female tissues (Fig. 1.2) [1715]. They begin life with two X chromosomes but typically lose an X during the first mitosis, so that one of the two zygotic nuclei becomes 1X [1695]. Because gender in flies is dictated by the number of Xs relative to the numbers of autosomes [817], the 1X nucleus – and the half of the body that it populates – becomes male. Sexual traits are expressed autonomously at a single-cell level because flies lack circulating sex hormones. The male/female boundary can be mapped throughout the cuticle (not just in dimorphic organs) by using recessive mutations to mark one of the Xs. The *yellow*^{LOF} mutation is often used because it turns the normally brown bristles (and cuticle) yellow [4101]. Such flies are useful for cell lineage analysis because any body part that develops clonally must come

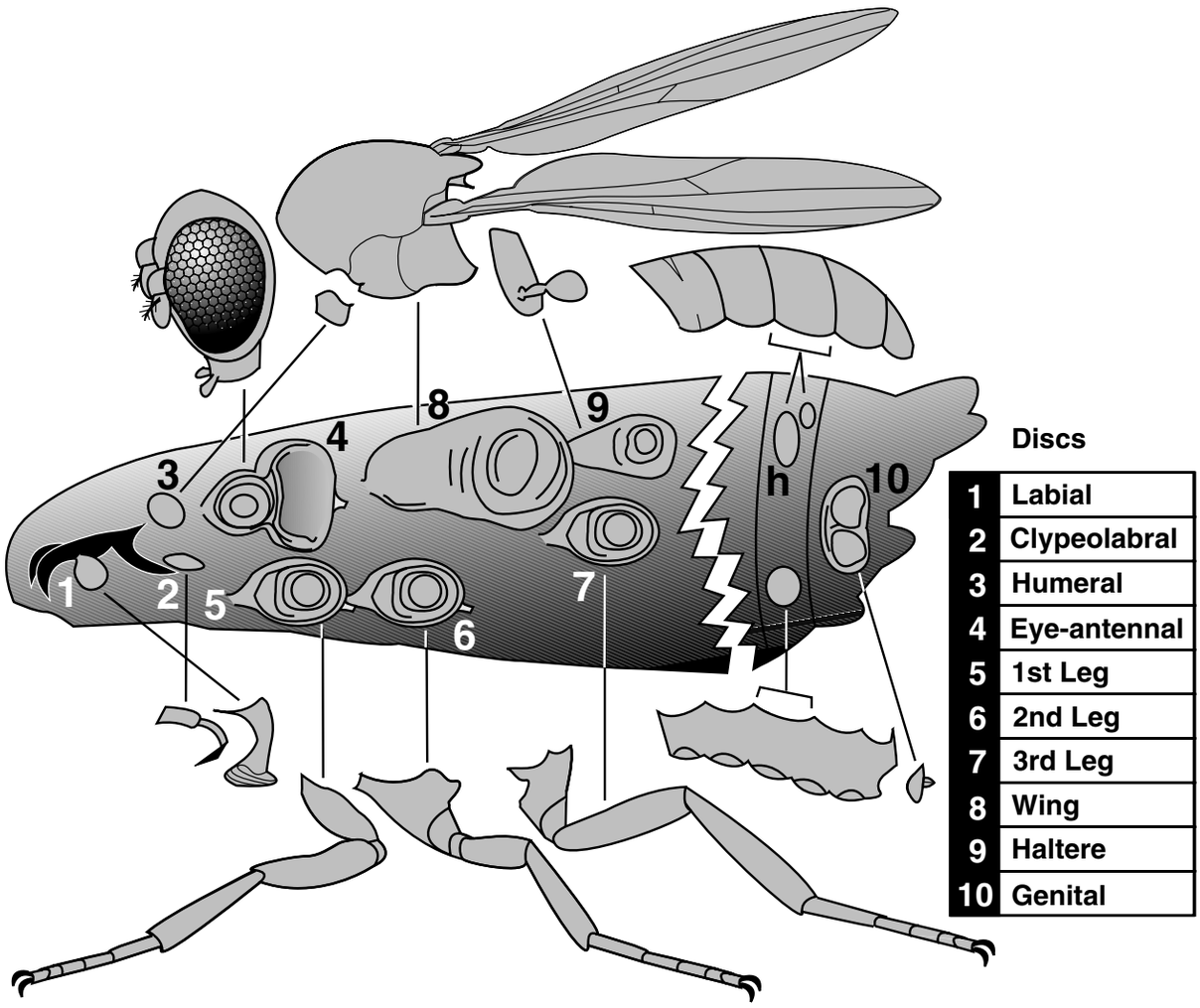


FIGURE 1.1. Imaginal discs and their cuticular products. The fly exterior is assembled from separate parts (like an automobile). The epidermis of the head and thorax come from 9 bilateral pairs of discs (one of each kind is shown), and genitalia come from a medial disc, so there are 19 discs total. Abdominal wall comes from histoblast nests (**h**): tergites from dorsal nests, and sternites and pleurae from ventral nests.

Discs are drawn to the same scale, and are oriented to display their mature shapes and folding. Placements are approximate. Clypeolabral and labial discs are attached to the pharyngeal skeleton (black hooks) [3285], while other discs adhere to other larval organs (not shown) [527,834,4565]. "Humeral" is synonymous with "dorsal prothoracic" disc. Bristles are omitted for clarity, and flank sclerites are simplified.

An adult fruit fly is ~3 mm long. Full-grown larvae are roughly twice that length [3421]. About half the larval midsection is omitted here. Adapted from [1739,4565].

Discs look more alike than the structures they produce. The same is true at the cell level, where discs are nearly indistinguishable by ordinary histology [3165,4424]. Even at the molecular level, different discs make virtually identical suites of proteins [1459,1611,3625,3756,3865], although amounts vary. The reason for these common features – as later chapters show – is that all discs use the same basic "toolkit" of molecules for intercellular signaling [662], although the circuitry (i.e., how those molecules interact) is tailored to the disc-specific patterns [1440].

from one single male or female progenitor cell and hence be purely yellow or brown.

Sturtevant discovered that cuticular derivatives of all the larger discs can be bisected by a yellow/brown boundary. Hence, these discs do not develop as clones. Subsequent studies found mosaicism in the smaller discs as well [1370, 2026, 2029, 2828]. By implication, each disc must come from ≥2 cells [2411]. In fact, when discs are first

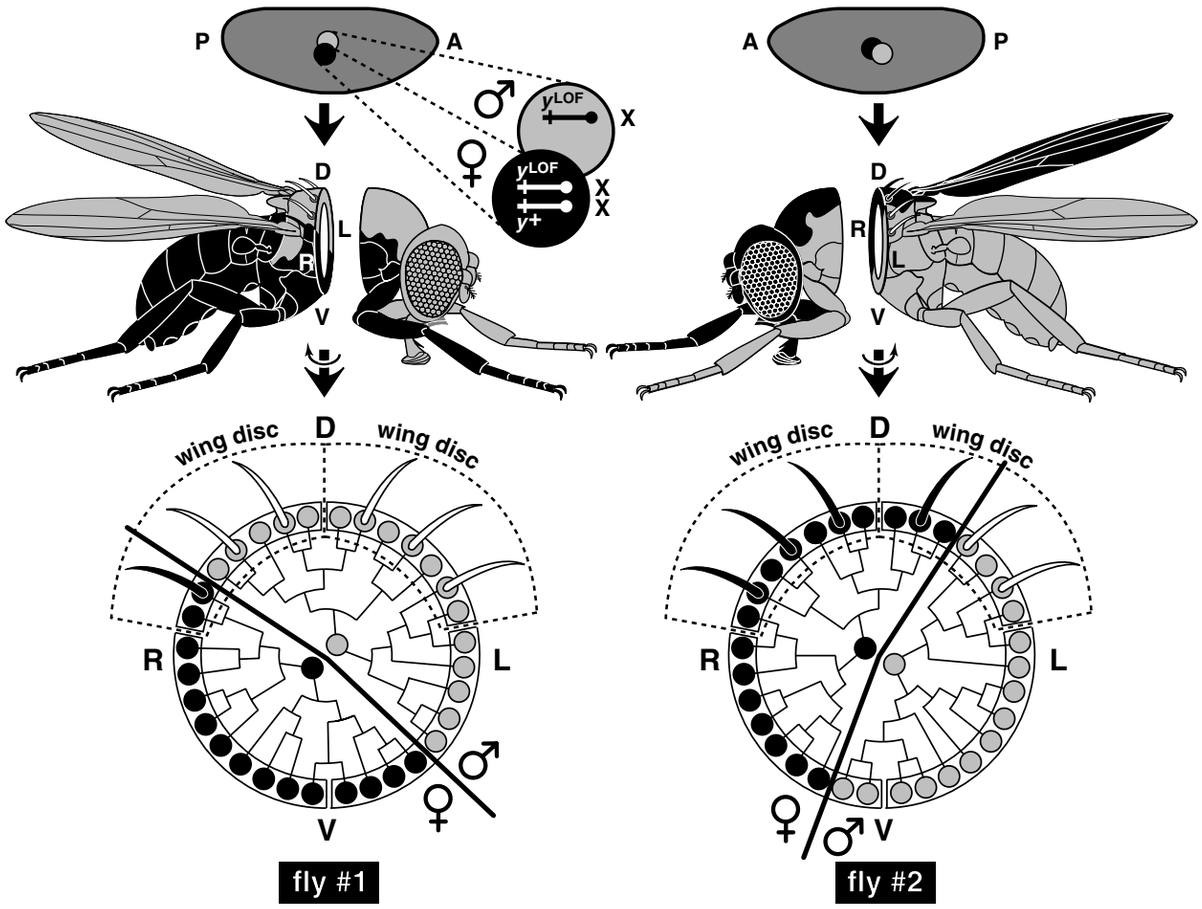


FIGURE 1.2. The nonclonal nature of fly development. The irrelevance of cell clones to pattern formation is seen in the piebald variegation of sexually mosaic "gynandromorphs" (middle panel) [1370,2026]. Such flies are typically half male (gray) and half female (black) [1715,2950]. They start life as a heterozygous female (2X) zygote but lose an X chromosome from one nucleus at the first mitosis to create a male (1X) clone (fly 1, top panel) [1695]. If the X that remains has the *yellow*^{LOF} (y^{LOF}) allele (enlarged gray circle), then the descendants of that nucleus will make yellow (instead of brown = wild-type) bristles or cuticle in the adult (fly 1, bottom panel).

The two embryos at the top of the figure (A, anterior; P, posterior) differ in the orientation of the first mitotic spindle [3274,4021]. This disparity causes the male/female boundary to trace different paths in the cuticle (middle panel) [4649,4652,4845].

The adults are bisected in the middle panel, and the cross-sections are turned ~90° to a frontal view in the bottom panel (D, dorsal; V, ventral; R, right; L, left). The outer ring of circles (nuclei) schematically represents the thoracic epidermis. The inscribed "tree" represents an imaginary series of mitoses (branch points) from the initial two nuclei to the adult epidermis. Bristle numbers and cell densities are drastically reduced for clarity.

If the wing disc (dashed outline) were a clone – i.e., derived from a single nucleus – then it should be purely yellow or brown because its progenitor nucleus must be one or the other. In actual gynandromorphs, however, the wing disc is often mosaic (R disc in fly 1 and L disc in fly 2), so it cannot be a clone. Moreover, the ability of the male/female boundaries to pass between any two landmarks (e.g., the different pairs of bristles in fly 1 vs. fly 2) argues that the patterns of cell lineage within the disc (inscribed trees) must also vary from fly to fly.

Overall, therefore, such flies reveal a fundamental uncoupling between pedigrees and patterning. This uncoupling is abstractly seen in the ability of the male/female "hour hands" to lie anywhere on the epidermal "clockface." The two flies shown here are only two examples from a large set of possibilities.

detectable histologically, each contains at least 10 cells (cf. Ch. 4). It is a quirk of history that the full import of Sturtevant's study was only realized 40 years later [119] when Antonio García-Bellido and John Merriam used Sturtevant's data to map the embryonic disc primordia [1370].

No part of a disc is a clone, except claws and tiny sense organs

Yellow/brown gynandromorphs are as eye-catching as a herd of Appaloosa horses because each individual has a unique pattern of colored patches (Fig. 1.2) [2026]. Their harlequin variegation is due to (1) the random orientation of the zygote's first mitotic division in all three dimensions from one individual to the next [3274, 4021] and (2) the tendency of sister nuclei to stay together during cleavage [4899]. The male/female line hence intersects the egg surface at random angles [4222, 4845], and the yellow/brown boundary should bisect any given area of the adult surface if a sufficiently large population is examined – *unless that area is delimited clonally*. Among the 96 specimens that Sturtevant analyzed, many groups of cuticular landmarks were divided by such boundaries. This "indeterminate" cell lineage was epitomized by two pairs of bristles that belong to the wing disc: the dorsocentrals and postalars. From one fly to the next, Sturtevant found that

> both dorsocentrals may be alike [i.e. both male or both female] but different from both postalars, or the posterior dorsocentral and posterior postalar may be alike but different from the corresponding anteriors, or any one of the four may be different from the other three. Such relations occur for any group of mesonotal bristles one examines. [4180]

Indeed, male/female boundaries meander relatively freely through every bristle array on the adult surface [1800, 2026, 3007, 3539, 4652]. Clearly, discs are not balkanized into subregions where individual cells obey commands such as *"Divide 'n' times and tell your descendants to make this part of the adult."* The only exceptions are (1) bristles and sensilla [3441] whose few component cells ($\leq$10) come from single "mother" cells and (2) claws [1356], which follow a similar developmental path [1587]. Additional instances are found in embryonic development – e.g., neural ganglia [627], muscle subtypes [250, 3684, 3698], and cardiac precursors [1339, 4194, 4547]. Wherever cell-type determination is uncoupled from cell lineage – as here in the case of large-scale patterning within discs – it must perforce rely on intercellular signaling [293, 354, 4727].

Cells belong to lineage "compartments"

Despite the rarity of rigid pedigrees in disc development, cells commonly obey looser edicts such as "*You may make any portion of region 'R', but nothing outside it*" [4671]. Regional limits of this kind were discovered in wing discs when marked cells were spurred to grow faster than background cells. Oversize anterior or posterior clones grew up to – but failed to cross – a boundary that roughly bisects the disc [1376, 1377], and analogous "compartments" were later found in halteres [1358, 1771], legs [1800, 2449, 4076], antennae [2931], genitalia [1107, 2028], and the proboscis [4144, 4145]. Compartments are essential for patterning (cf. Ch. 4 *ff*), but their lineage constraints per se are not [754, 2428, 2448, 2677, 4491]. Hence, the existence of these clans does not negate the "**Proximity vs. Pedigree Rule**" [3445] enunciated above. Put simply, this rule asserts that cells select fates based on input from peers, not parents [354, 526, 1808].

CHAPTER TWO

The Bristle

Tactile stimuli are hard for arthropods to detect through the armor of their rigid exoskeleton [1666, 3582]. To solve this problem, flies use bristles (Fig. 2.1). When a bristle is deflected, the pivoting of the shaft in its socket deforms the dendrite of a neuron attached to the shaft's base [789, 1352, 2174, 2787]. The resulting depolarization sends an action potential to the central nervous system (CNS) [1118, 2173, 2196, 4527]. Flies can pinpoint sensations because axons from different bristles get "wired" to different CNS target cells during metamorphosis, although much remains to be learned about the topology of these neurosensory maps (cf. Ch. 6).

Mechanosensory bristles are formed by 5 cells: 2 superficial cells that secrete cuticle (the shaft and socket cells) and 3 subepidermal cells that do not (the neuron, sheath, and glial cells) [2475, 3351, 3552, 3832, 4531]. These 5 cells descend from a "sensory organ precursor" (SOP). The SOP divides to produce one daughter (IIa) that yields the outer cells, and another (IIb) that yields the inner cells [1447, 1741, 1925]. The sheath cell wraps the neuron's dendrite [602, 789, 3351], while the glial cell wraps the axon [2173]. A sixth cell – the "bract cell" – is found in association with bristles on the distal leg and proximal wing [524, 1714, 1808]. It secretes a thickened hair ("bract") that is pigmented like the bristle shaft but much smaller [3362, 3421]. The bract cell is not part of the SOP clone [1808]. The way in which it is recruited from epidermal cells is discussed later.

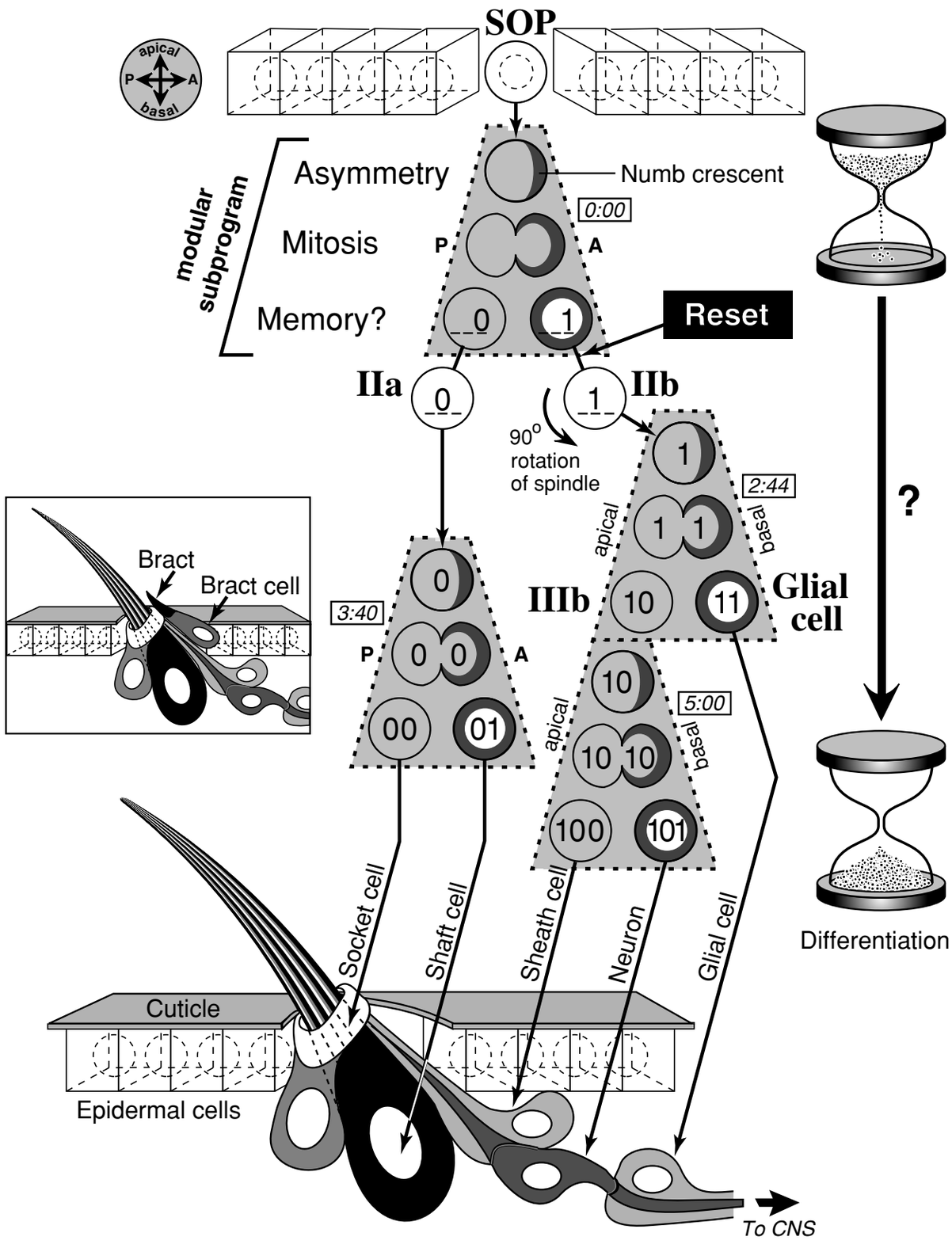
Until 1999, the glial cell's origin was obscure [1463, 1465, 1741, 1925], and only the shaft, socket, sheath, and neuron were considered to comprise the SOP clone. In 1999, a debate about the sequence of bristle cell mitoses [2680, 3550] prompted a reinvestigation of the mitoses themselves [1447, 3549], whereupon a new mitosis was discovered. It had hitherto been overlooked because the glial cell is small and migrates away from its birthplace. Pre-1999 models are being revised to include this amendment [2382].

Chemosensory bristles have all the components of a mechanosensory bristle plus 4 extra neurons, whose dendrites project to a pore at the shaft's tip [1741, 3061, 3529, 4841] where they detect chemicals (Fig. 2.8) [3835]. Strangely, such bristles (on the legs and wings at least) are also photosensitive, with independently entrainable circadian clocks [2333, 3327, 3401]. Aside from sensory modality [3005], fly bristles also vary in size, shape, pigmentation, and pattern.

Bristles are intriguing not only because their stereotyped mitoses violate the general rule of indeterminate lineage (cf. Ch. 1), but also because they encapsulate the problem of differentiation (how do cells acquire differences?) [2424, 2577, 4658]. In theory, the instructions for assigning fates could be unequally inherited from the SOP, with no need for cross-talk among descendants. According to this "**Obey Your Mother! Model**," bristle cells adopt fates based on cues inherited from their mothers. The main cue appears to be the presence or absence of a membrane-associated protein called "Numb." Numb has all the features expected for a heritable determinant of cell fate.

Numb segregates asymmetrically and dictates bristle cell fates

The gene *numb* was isolated in a screen for mutations affecting the embryonic peripheral nervous system (PNS) [4417]. In a seminal 1994 article that provided the key to deciphering bristle differentiation, Michelle Rhyu *et al.*



in San Francisco reported that *numb* mutations also affect adult bristles [3579] and, more important, that Numb protein is distributed unequally during SOP divisions. Indeed, this was the first gene product in flies ever shown to segregate asymmetrically in mitosis, although others soon followed [2021].

Within the SOP lineage, 4 cells inherit Numb (IIb, shaft cell, glial cell, neuron; Fig. 2.1) [1447, 3579, 4542] while 4 do not (IIa, socket cell, IIIb, sheath cell), and mutant defects are generally consistent with this parceling. Thus, $numb^{null}$ mutations cause SOPs to produce 4 outer cells and no inner ones – implying that IIb adopts a IIa fate – and the outer cells are often all sockets, so a shaft-to-socket transformation must also be involved. A third conversion (neuron-to-sheath) occurs in hypomorphs [4542]. Collectively, these phenotypes imply a fate-assigning role for *numb* at every mitosis in the lineage, with the possible exception of the glia-producing IIb mitosis, which, as mentioned above, has only recently begun to be studied.

The history of a cell's Numb states can be denoted by the left-to-right order of digits in a binary code (Fig. 2.1), where "1" signifies Numb's presence and "0" its absence. Thus, the various sister cells in the SOP lineage would have the following paired codes:

IIa (0) vs. IIb (1).
Socket (00) vs. shaft (01).
IIIb (10) vs. glial cell (11).
Sheath (100) vs. neuron (101).

From the standpoint of a strict "**Coding Model**," the code would be causal. That is, a bristle cell's fate would be dictated by the series of Numb states (0 or 1) experienced by its ancestors. This code would explain the null phenotype where all cells assume a 00 (socket) state, and it would also explain the hypomorphic condition where neurons (101) switch to sheaths (100). To wit, leaky Numb levels might be high enough to let IIb attain its "1" state but not to push neurons into their later "1" state.

One test of this model would be to overexpress *numb*. Flooding the lineage with Numb protein should raise all "0" states to "1" and cause all cells to differentiate as glia (11). When *UAS-numb* is driven by a *Gal4* transgene expressed in SOPs, no clusters of 4 glial cells were reported [4542]. The most extreme defect was a 4-neuron trait where IIa likely became IIb (0 ➞ 1) and sheath cells became neurons (100 ➞ 101). Milder abnormalities were also seen, including "2 sheaths: 2 neurons" (0 ➞ 1 but not 100 ➞ 101) and duplicated shafts (00 ➞ 01 but not 0 ➞ 1). Overall, the data agree with the model, although the failure to force cells into a glial fate is problematic. Perhaps the excess Numb cannot prevent Numb's level from being reset to "0" in IIb (Fig. 2.1).

Additional support for the model comes from flies carrying a *hs-numb* construct (*numb* joined to a heat-shock promoter). When such flies are heat-shocked around the time of SOP mitoses, they display "2 sheaths: 2 neurons" as well as "2 shafts, sheath, neuron" (socket-to-shaft conversion) and "socket, shaft, 2 neurons" (sheath-to-neuron). These defects are explicable by the forced presence of Numb in the IIa (0 ➞ 1), socket (00 ➞ 01), or sheath (100 ➞ 101) cell [3579]. Four-neuron

FIGURE 2.1. Development of a mechanosensory bristle from a sensory organ precursor (SOP). Compass (upper left) gives initial directions (A, anterior; P, posterior). Times (hours: minutes at 23°C) are for microchaete mitoses on the notum but are similar for other bristles [1447].

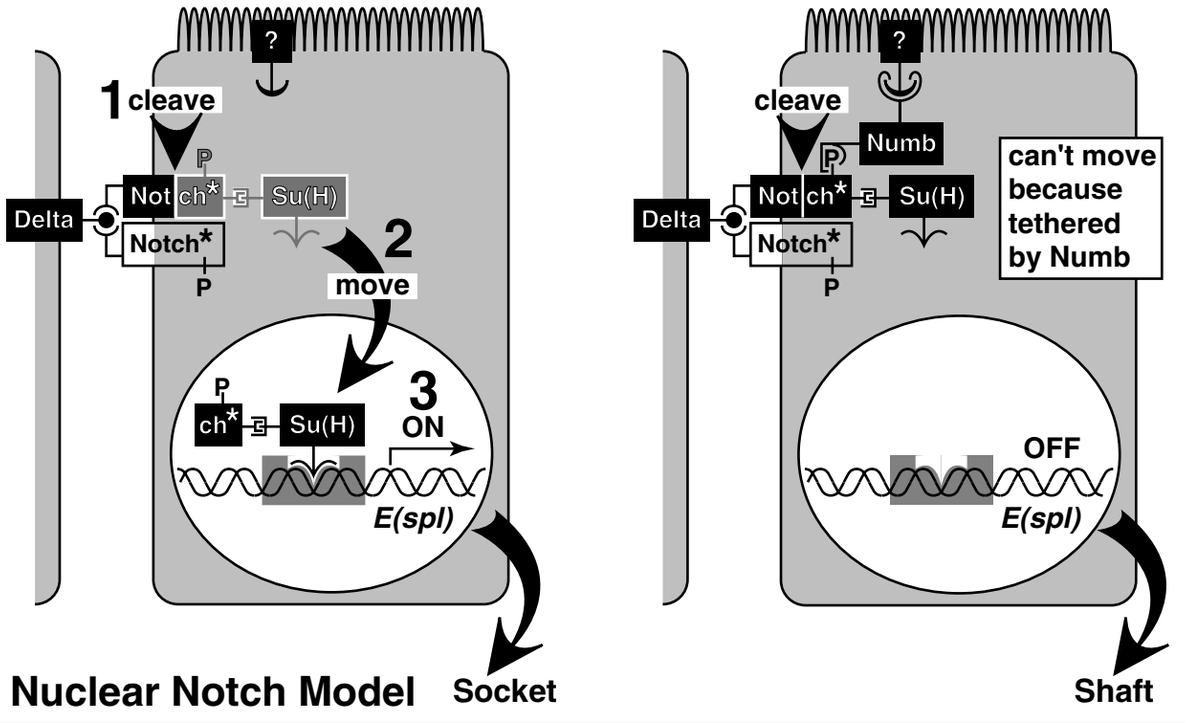
The SOP arises from an ordinary epithelial cell. It starts to divide (at ~16 h after pupariation) to form IIa and IIb. IIa's daughters will make a socket and shaft. IIb's daughters are IIIb and a glial cell. The glial cell is smaller and buds off basally in the manner of a CNS neuroblast division [1073, 1740]. IIIb divides to form a sheath cell and neuron. Some bristles have a thick hair ("bract") atop their sockets (inset), which is made by a clonally unrelated cell.

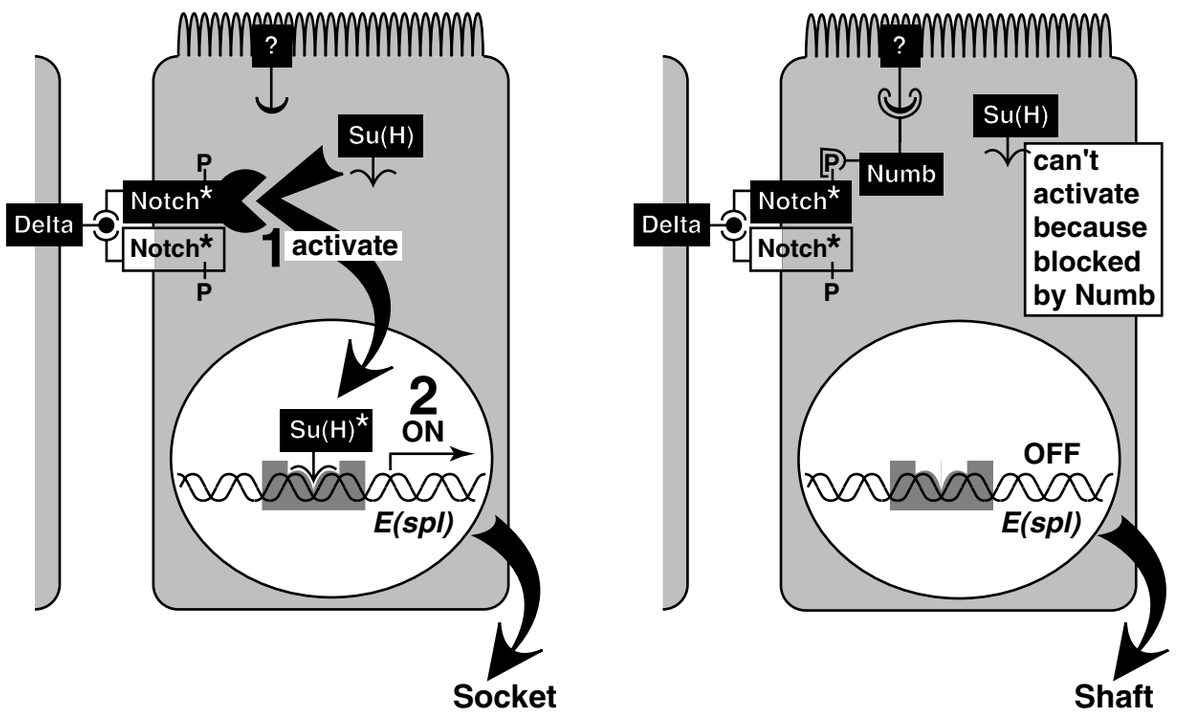
Each mitosis obeys stereotyped steps (dashed trapezoid) that comprise a modular subprogram: (1) Numb localizes to one side of the cell cortex (crescent), (2) segregates to one daughter, and (3) alters cell fate. Letting 1 and 0 signify Numb's presence or absence, each cell can acquire a unique code if it "remembers" its former Numb states. Imaginary memory registers (underlined spaces) are shown for a few cells, with left-to-right order recording successively later states.

For such a binary code to work, IIb must eliminate ("reset") Numb before dividing. When SOPs are prevented from dividing, they become neurons [1743]. This result has been interpreted as a default condition, but it may instead reflect persistence of Numb: the continual presence of Numb should lead to a "nonsense" code (111) that might be interpreted as "neuron" (101). The mechanism whereby cells remember former Numb states is unknown.

Timing and branching of the pedigree are as per [1447, 3549]. Other details are based on [1449, 1741, 1808, 3579]. See [3195] for lineage comparisons with other sensory organs.

N.B.: Grooves are absent from some bristles (e.g., sex comb teeth [1714]). Epidermal cells are sometimes aligned with this degree of precision [2388], although they need not be. Chemosensory bristles have 4 additional neurons (cf. Fig. 2.8) [4125], and their SOPs obey a different lineage [3529]. See also App. 7.





phenotypes were not observed, probably because pulses were too short to affect all three rounds of (asynchronous) mitoses.

If Numb were a traditional "cytoplasmic determinant," then it would specify only one type of tissue or cell [1904]. On the contrary, it marks 4 different cells in the SOP lineage. Moreover, it plays similar roles in sense organs of the larval PNS [3579, 4417], in neuroblasts of the embryonic CNS [2451, 3579, 4028, 4523], in cardiac cell progenitors [1339, 4194, 4547], in sibling founder cells of larval vs. adult muscles [653, 3684], and in muscle subtype determination [251, 910, 3263, 3687]. Thus, its role transcends histotype.

Evidently, Numb functions as a versatile switch that enables daughter cells to become different from one another, regardless of what those differences may be [831, 1761, 3263, 3579, 4875]. As a binary digit ("bit"), Numb is the best example ever adduced that flies can use abstract symbols for instructions just as computers employ machine language. As explained below, this "**Numb Epiphany**" of 1994 is not only helping to elucidate how genes can work as switches, but it is also revealing how an intrinsic mechanism of fate specification can dovetail with an extrinsic pathway of intercellular signaling.

Delta needs to activate Notch, but not as a signal per se

Although the Numb code should be sufficient for assigning all fates, some cell interactions have also been implicated. The 4-neuron trait that is caused by gain-of-function (GOF) *numb* manipulations is also seen with loss-of-function (LOF) mutations in *Delta* (*Dl*) and *Notch* (*N*) [1742, 3272]. Because *Dl* and *N* mediate "sibling rivalries," whereby equivalent cells become different (cf. Fig. 3.6) [2222, 3022], they could – in theory – create binary codes by refereeing a series of bouts (winner = 1; loser = 0) without relying on cell pedigrees at all [1614].

Might fates be computed by either lineage (via Numb) or signaling (via Dl and N), with one agent assuring success if the other fails? No, because such redundancy would imply that phenotypes should be wild-type unless both strategies fail, but (as stated above) fates can be altered by single LOF mutations in *numb*, *Dl*, or *N*. Rather, it seems that the two devices are connected in series, not in parallel.

Dl and N are transmembrane proteins that interact as ligand (signal) and receptor (receiver), respectively [1204, 2626]. When a N-expressing cell contacts a Dl-expressing cell, N is activated by dimerization [3022] or oligomerization [2209, 2299]. Activation causes N's intracellular domain ("N-intra") to detach from the membrane and go to the nucleus, where it stimulates transcription of target genes [4155]. Numb may block signaling by tethering N-intra to the cortex (Fig. 2.2), thus keeping it from reaching its targets. Enough Numb would normally be present to sequester all N, although an artificial excess of N could escape Numb's grasp and cause the kinds of N^{GOF} phenotypes that are seen [1307, 1651].

The need for ligand may suggest extrinsic signals, but there cannot be any instructive (ON/OFF) signaling per se [1433] because Dl is expressed at equal levels in IIa, IIb, and surrounding nonbristle cells [3270]. Evidently, Dl plays only a permissive role, essentially like a seaman sending Morse code by using a shutter (Numb) to blink a light (Dl-N) that stays ON. This "**Blinker Model**" supposes that Dl's job is merely to keep N active so that the nucleus only gets a "N = OFF" signal when Numb is present. Mosaic analyses suggest that the SOP descendants themselves supply one another with the ligands for N stimulation, with no reliance on surrounding epidermal cells [4859]. This intrabristle cross-talk has been confirmed in an interesting experiment. When Dl is overexpressed in the neuron, the adjacent shaft cell

FIGURE 2.2. Models for Notch signaling and its blockage by Numb. Black rectangles are proteins, and connecting "wires" are binding sites. Contact with Delta ligand on a neighbor's surface activates (asterisk) the Notch receptor, possibly by dimerization (partner outlined) [3022]. Cells that lack Numb (left) can relay the signal to its nucleus, while those that express Numb (right) cannot.

The models differ in how Numb stops the signal. In the **Nuclear Notch Model** (above the line) [1307, 1448, 1651, 2299, 4027, 4244, 4542], Numb stops Notch from leaving its roost (ghost image) by anchoring it to the membrane [2267] via an unknown linker ("?" = possibly Partner of Numb [2609]). In the **Catalysis Model** (below the line) [112, 132, 1131, 3022, 4244], Numb blocks an active site for Su(H) activation (covalent modification?).

Numb is shown binding Notch at a phosphotyrosine (P), but Numb's PTB domain is unusual and may not need a phosphate [2530, 4789], and Notch is only known to have phosphoserines [2209]. Notch resides in the apicolateral membrane [184, 1203, 1448, 2070]. The cell's apex is carpeted with microvilli. Su(H) can activate transcription (right-angle arrow) of *E(spl)* (a.k.a. "*m8*"; cf. Fig. 2.4) by binding its promoter (gray rectangle), but *E(spl)* may not dictate bristle cell fates, nor is *Su(H)* needed for signal relay in neurons or sheath cells (see text). Estimates are that a signal at the membrane takes ~20–90 min to cause detectable changes in target gene expression [184]. See also App. 7.

transforms into a socket cell (Fig. 2.7c) [2008]. Clearly, Numb's lock on the Notch pathway can be artificially overridden by excess Dl.

In contrast to the Blinker Model, the popular view has been that Numb merely biases Dl-N contests [1613, 2019, 2021, 2222, 3437], rather than being the sole deciding factor. Yuh Nung Jan and Lily Yeh Jan, who pioneered this field, advocated this "**Bias Model**" but recognized an inherent paradox: because one sister cell should win every contest (with or without a Numb handicap) the *numb*^{null} phenotype should be wild-type, but it is not (and the same dilemma applies in the CNS [552]). To explain why, they invoked time constraints [2020]:

> We think that . . . an intrinsic mechanism utilizing numb protein is superimposed on the Notch/Delta system to bias the competition. . . . We speculate that this Notch/Delta system is not sufficiently reliable to ensure that the two cells always acquire two different fates in the allotted time. (In the case of IIa vs. IIb fates, the time window is less than 2 hr.) . . . This hypothesis could explain the variable phenotype resulting from complete loss of *numb* function. In *numb*^{null} clones some sensory bristles show the severe phenotype of having four socket cells, whereas other sensory bristles develop normally. Our interpretation is that, in the absence of *numb*, the Notch/Delta system still operates, but is not sufficiently reliable. . . . Some sensory bristle cells were able to finish the competition and form normal sensory bristles with four distinct fates, whereas others were unable to do so.

The Bias Model predicts that contests will end in Dl-rich/Dl-poor (winner/loser) cell pairs. On the contrary, only Dl-equivalent pairs are detected in wild-type flies [3270]. Rejecting the Bias Model in favor of the Numb-dictated "Obey Your Mother!" Model still leaves the question of why all bristle sites in *numb*^{null} clones do not have a 4-socket phenotype [2019]. Perhaps the normally dormant Dl-N rivalry mechanism has been awakened in these clones, in which case they should manifest Dl-rich/Dl-poor cell pairs (a testable prediction). Alternatively, unknown asymmetries may be augmenting Numb's function (i.e., a *partial* redundancy). Either way, Numb's control over N begs the evolutionary question: how did a heritable determinant (Numb) "hijack" an intercellular signaling pathway (Dl-N)?

Amnesic cells can use sequential gating to simulate a binary code

If Numb is the bit in the bristle formula, then how do cells interpret 2- and 3-bit "words" for the various cell types? A simple ratcheting mechanism, whereby cells count how many times they have been "1," cannot suffice because in that case "01" and "10" would be synonyms. It would seem that cells must use some sort of combinatorial code where genes aside from *numb* are used for recording previous Numb states. Figure 2.1 illustrates such a Coding Model.

Do any known genes behave like a primary memory register – namely, their mutant alleles convert IIa into IIb (or vice versa) without switching any subsequent states? Among the genes whose mutant phenotypes connote a IIa-IIb switch, only *Bearded* (*Brd*) lacks later effects (App. 3) [2500]. Its GOF phenotype consists of neurons and sheath cells without shafts or sockets – indicative of a transformation of IIa into IIb. Thus, *Brd* could store the outcome of the first mitosis. (N would turn *Brd* OFF in IIa.) *Brd*^{null} mutants look wild-type [2500], but this impotence is attributable to redundant paralogs [2382].

There is another way of thinking about Numb's mode of action that does not involve memory genes per se. To wit, Numb's first state (0 or 1) might simply "gate" IIa and IIb into divergent signal transduction pathways (STPs), so that the second Numb signal (0 or 1) is interpreted differently by IIa daughters (STP 1) vs. IIb daughters (STP 2). According to this "**Gating Model**," genes that act only in the IIa STP should interconvert shafts and sockets when mutated, but should have no effect on neurons, sheath, or glial cells (IIb descendants), and separate sets of STP genes would operate exclusively in the IIb and IIIb sublineages.

Indeed, *Suppressor of Hairless* appears to be a IIa-specific STP gene. Null *Su(H)* mutations suppress only part of the phenotype caused by *numb*^{LOF} – namely, the shaft-to-socket switch but not the neuron-to-sheath switch – implying that *Su(H)* is only needed in the IIa lineage [4542]. This conclusion is bolstered by the ability of excess Su(H) to transform IIa (shaft-to-socket) but not IIIb daughters [200, 3827, 4542]. Su(H) is detected in both the IIa and IIIb lineages, but its level is highest in the socket cell (as are *Su(H)* transcripts [3826]) – a IIa daughter [1448]. Su(H) moves from the socket cell's cytoplasm to its nucleus when N is activated [1448] – precisely the behavior expected for a messenger molecule [1269, 1307]. Su(H) can bind both to N (signal acquisition?) [1269] and to DNA sites (signal delivery?) upstream of genes in the *Enhancer of split* Complex [1131, 2453], which may control bristle cell fates (but see below). Thus, Su(H) has not only the phenotypic properties of a IIa STP agent, but also the histological hallmarks.