The spatiotemporal order of signaling events unveils the logic of development signalling

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1. Supplementary Introduction

In the imaginal eye disc, cell cycle regulation, cell shape change, and cell fate determination are tightly coordinated in a band of cells that form the morphogenetic furrow (MF) (Frankfort and Mardon, 2002; Silver and Rebay, 2005; Treisman and Heberlein, 1998). Anterior to the MF, cell fates are undetermined and the proneural gene *atonal* (*ato*) is repressed by the antineural Hairy and Extramacrochaete (Emc) (Treisman and Heberlein, 1998). Posterior to the MF, R2/R5 express Hedgehog (Hh) that diffuses anteriorly over several ommatidia to cause active Cubitus interruptus (CiA) accumulation, and R8 expresses Ato that activates Rough (Ro) in neighboring cells to repress *ato* expression (Baonza et al., 2001; Dokucu et al., 1996; Dominguez and Hafen, 1997). CiA induces *ato* and *decapentaplegic* (*dpp*) expression in cells within and anterior to the MF, but is ubiquitinated by Hh-induced MATH- and BTB-domain containing protein (HIB) in cells posterior to the MF (Dominguez and Hafen, 1997; Zhang et al., 2006). Dpp signaling is required for cell cycle arrest (before the MF), but not for R8 determination (in the MF) (Fu and Baker, 2003). Wingless (Wnt) signaling seems important mainly in the peripheral area of the eye disk (Lee and Treisman, 2001). When the Ato level exceeds a threshold Ato autoactivation occurs, which makes the cell an R8 (Sun et al., 1998). Ato induces *delta* in all cells, *scabrous* (*sca*) in proneural cells, and *rhomboid* (*rho*) in R8 precursors, which activate Notch and EGFR signaling respectively within the MF (Chen and Chien, 1999; Li and Baker, 2001; Powell et al., 2001; Spencer et al., 1998). In proneural cells Sca competes with Delta to bind to Notch, and MAPK is active. In intervening cells Delta/Notch binding produces Notch intracellular domain (NICD). Repressed by Rho and activated by a diffusible inhibitory factor downstream to EGFR signaling (Chen and Chien, 1999; Dokucu et al., 1996; Li and Baker, 2001; Powell et al., 2001; Spencer et al., 1998), *ro* is expressed in cells to repress *ato*. Both NICD and Ro down regulate *ato* expression in cells that are not R8 precursors (Baonza et al., 2001; Dokucu et al., 1996; Powell et al., 2001) (Figure 1B).

2. Supplementary Methods

A. Basic forms of equations

The generic formula for each molecular species U with concentration $[U]$ can be described by (von Dassow et al., 2000)

$$\frac{d[U]}{dt} = \text{synthesis} - \text{decay} \pm \text{transformation} \pm \text{transport} \tag{A1}$$

Taking the mRNA *hh* and protein Hh as example, the synthesis of *hh* activated by Pointed (Pnt) is

$$\frac{d[hh]}{dt} = R_{hh} \cdot \left(\frac{[Pnt]^h}{\chi_{Pnt\_hh}{}^h + [Pnt]^h} \right), \tag{A2}$$

where R_{hh} is the maximum transcription rate, and the synthesis of Hh is

$$\frac{d[Hh]}{dt} = P_{Hh} \cdot [hh], \tag{A3}$$

where P_{Hh} is the maximum translation rate. As both the protein and mRNA follow the first-order decay, we also have

$$\frac{d[hh]}{dt} = -\mu_{hh}[hh] = -\frac{\ln 2}{H_{hh}}[hh], \tag{A4}$$

$$\frac{d[Hh]}{dt} = -\mu_{Hh}[Hh] = -\frac{\ln 2}{H_{Hh}}[Hh]. \tag{A5}$$

μ_{hh} and μ_{Hh} are decay rate and H_{hh} and H_{Hh} are half-life of hh and Hh respectively.

Two kinds of protein transformation are included in our model: (1) dimerization (including ligand/receptor binding) and (2) phosphorylation (including cleavage). The rate of dimerization of U and V is described by

$$k_{uv} \cdot [U] \cdot [V], \tag{A6}$$

with k_{uv} being a parameter describing the affinity. The phosphorylation or cleavage of U by V depends nonlinearly on the concentration of V and is described by

$$c_{vu} \cdot [U] \cdot \left(\frac{[V]^h}{\chi_{V\_U}{}^h + [V]^h} \right), \tag{A7}$$

where c_{vu} is the maximal phosphorylation/cleavage rate.

Unless specifically noted, a large Hill coefficient is used in all transcriptions, a medium one in all transformations, and a small one for Notch/Delta lateral inhibition.

Only one kind of molecular transport is involved, i.e., intercellular diffusion, which is described by

$$\frac{dU_{[0,0]}}{dt} = D\left(\frac{U_{[1,0]} + U_{[-1,0]} + U_{[0,1]} + U_{[0,-1]} - 4U_{[0,0]}}{L^2} \right) = D\nabla^2 U_{[0,0]}, \tag{A8}$$

where D is the diffusion coefficient, L is the cell length and the 5-point stencil on a square lattice is used. We denote this discrete diffusion operator by ∇^2.

B. Nondimensionalization

We use a simple representation of the Hh signalling system to demonstrate the nondimensionalization of equations. The most basic representation of the Hh pathway comprises one ligand (Hh), one receptor (Ptc) and one effector (Ci). Ptc is either free (PtcF) or Hh-bound (PtcB), and Ci is in either the active form (CiA) or the repressive form (CiR). To

describe *hh* activation by Pnt, Hh diffusion into neighbouring cells, Hh binding to PtcF, and PtcB stopping the cleavage of CiA into CiR, the following equations are formalized

$$\frac{d[hh]}{dT} = R_{hh}\left(\frac{[Pnt]^h}{\chi_{Pnt\_hh}{}^h + [Pnt]^h}\right) - \mu_{hh}[hh] \tag{B1}$$

$$\frac{d[Hh]}{dT} = D\nabla^2[Hh] + P_{Hh}[hh] - \mu_{Hh}[Hh] - k_{HhSmo}[Hh][PtcF] \tag{B2}$$

$$\frac{d[PtcF]}{dT} = P_{Ptc}[ptc] - \mu_{PtcF}[PtcF] - k_{HhPtc}\cdot[Hh]\cdot[PtcF] \tag{B3}$$

$$\frac{d[PtcB]}{dT} = k_{HhPtc}[Hh][PtcF] - \mu_{PtcB}[PtcB] \tag{B4}$$

$$\frac{d[CiA]}{dT} = P_{CiA}[ci] - \mu_{CiA}[CiA] - c_{CiA}\cdot[CiA]\cdot\left(\frac{\chi_{Ptc\_CiA}{}^h}{\chi_{Ptc\_CiA}{}^h + [PtcB]^h}\right) \tag{B5}$$

$$\frac{d[CiR]}{dT} = -\mu_{CiR}[CiR] + c_{CiA}\cdot[CiA]\cdot\left(\frac{\chi_{Ptc\_CiA}{}^h}{\chi_{Ptc\_CiA}{}^h + [PtcB]^h}\right), \tag{B6}$$

where *T* is the (dimensional) time variable.

At the steady state of *hh* transcription where $\left(\frac{[Pnt]^h}{\chi_{Pnt\_hh}{}^h + [Pnt]^h}\right) \cong 1.0$, *hh* reaches its maximal concentration $[hh]_0$ and Eqn (D1) becomes

$$\frac{R_{hh}}{\mu_{hh}} = [hh]_0. \tag{B7}$$

When both *hh* transcription and translation reaches the steady state where the maximal *Hh* concentration is $[Hh]_0$, if not considering *Hh* and *PtcF* binding, we have

$$P_{Hh}[hh]_0 = \mu_{Hh}[Hh]_0,$$

which gives

$$\frac{P_{Hh}}{\mu_{Hh}}\frac{R_{hh}}{\mu_{hh}} = \frac{P_{Hh}}{\mu_{Hh}}[hh]_0 = [Hh]_0. \tag{B8}$$

To nondimensionalize these equations we introduce the substitution

$$N = \frac{[N] - N^*}{\Delta N} \tag{B9}$$

for each dependent variable *N* and always let N^*=0. To scale [*N*] from [0, N_0] in the dimensional system into [0, 1] in the nondimensional system, we let

$$\Delta N = N_0$$

so that when $[N]=N_0$ we have

$$N = \frac{[N]}{\Delta N} = \frac{N_0}{\Delta N} = \frac{N_0}{N_0} = 1$$

Similarly we introduce the substitution

$$t = \frac{T - t^*}{\Delta t} \tag{B10}$$

for the independent time variable *T* and let t^*=0 and $\Delta t = T_0$. Finally we stipulate

$$\chi_{u\_v} = \varphi_{u\_v}[N]_0 \tag{B11}$$

With these substitutions, rearranging (D1)-(D6) yields

$$\frac{dhh}{dt} = \frac{T_0 \ln 2}{H_{hh}}\left(\frac{Pnt^h}{\varphi_{Pnt\_hh}{}^h + Pnt^h} - hh\right) \tag{B1'}$$

$$\frac{dHh}{dt} = T_0 D\nabla^2 Hh + \frac{T_0 \ln 2}{H_{Hh}}\left(hh - Hh\right) - T_0 k_{HhPtc}[PtcF]_0 \cdot Hh \cdot PtcF \quad \text{(B2')}$$

$$\frac{d\ PtcF}{d\,t} = \frac{T_0 \ln 2}{H_{Ptc}}(ptc - PtcF) - T_0 k_{HhPtc} \cdot [Hh]_0 Hh \cdot PtcF \quad \text{(B3')}$$

$$\frac{dPtcB}{dt} = \frac{T_0 \ln 2}{H_{PtcB}}\left(- PtcB\right) + T_0 k_{HhPtc}[Hh]_0 \cdot Hh \cdot PtcF \quad \text{(B4')}$$

$$\frac{dCiA}{dt} = \frac{T_0 \ln 2}{H_{CiA}}\left(ci - CiA\right) - T_0 \cdot c_{CiA} \cdot CiA \cdot \left(\frac{\varphi_{Ptc\_CiA}{}^h}{\varphi_{Ptc\_CiA}{}^h + PtcB^h}\right) \quad \text{(B5')}$$

$$\frac{d\ CiR}{d\,t} = \frac{T_0 \ln 2}{H_{CiR}}\left(-CiR\right) + T_0 \cdot c_{CiA} \cdot CiA \cdot \left(\frac{\varphi_{Ptc\_CiA}{}^h}{\varphi_{Ptc\_CiA}{}^h + PtcB^h}\right), \quad \text{(B6')}$$

where we have rewritten the linear degradation rates μ_U in terms of half lives H_U using

$$\mu_U = \frac{\ln 2}{H_U}.$$

We assume T_0=1.0, and since we do not have explicit values for any maximal molecular concentration in the eye disc, we also set $[PtcB]_0 = [PtcF]_0 = [Hh]_0 = 1.0$. Now Eqn (D1') to (D6') become

$$\frac{dhh}{dt} = \frac{\ln 2}{H_{hh}}\left(\frac{Pnt^h}{\varphi_{Pnt\_hh}{}^h + Pnt^h} - hh\right) \quad \text{(B1'')}$$

$$\frac{dHh}{dt} = D\nabla^2 Hh + \frac{\ln 2}{H_{Hh}}\left(hh - Hh\right) - k_{HhPtc} \cdot Hh \cdot PtcF \quad \text{(B2'')}$$

$$\frac{dPtcF}{d\,t} = \frac{\ln 2}{H_{Ptc}}(ptc - PtcF) - k_{HhPtc} \cdot Hh \cdot PtcF \quad \text{(B3'')}$$

$$\frac{dPtcB}{dt} = \frac{\ln 2}{H_{PtcB}}\left(- PtcB\right) + k_{HhPtc} \cdot Hh \cdot PtcF \quad \text{(B4'')}$$

$$\frac{dCiA}{dt} = \frac{\ln 2}{H_{CiA}}\left(ci - CiA\right) - c_{CiA} \cdot CiA \cdot \left(\frac{\varphi_{Ptc\_CiA}{}^h}{\varphi_{Ptc\_CiA}{}^h + PtcB^h}\right) \quad \text{(B5'')}$$

$$\frac{dCiR}{dt} = \frac{\ln 2}{H_{CiR}}\left(-CiR\right) + c_{CiA} \cdot CiA \cdot \left(\frac{\varphi_{Ptc\_CiA}{}^h}{\varphi_{Ptc\_CiA}{}^h + PtcB^h}\right) \quad \text{(B6'')}$$

C. Combinatorial control of transcription

The activation or inhibition of transcription of a gene by an activating or repressing transcription factor (TF) is described by the Hill function

$$\Phi = \left(\frac{[TF]^h}{\chi_{TF}{}^h + [TF]^h}\right) \quad \text{(C1)}$$

or

$$\Psi = \left(\frac{\chi_{TF}{}^h}{\chi_{TF}{}^h + [TF]^h}\right), \quad \text{(C2)}$$

respectively, where [*TF*] is the concentration of the TF. χ_{TF}, which we refer to as the half-maximal activation/repression coefficient, is the concentration of TF at which the gene is half-maximally activated or repressed, and *h,* the Hill coefficient, is the parameter that determines the 'steepness' of the Hill function (i.e. the sensitivity of the transcriptional regulation). An

enhancer can be bound by multiple activating and/or repressing TFs. We assume a successful activation of an enhancer requires the presence of all activating TFs and the absence of all inhibiting TFs. This AND condition stipulates the following combinatorial control

$$\prod \Phi_i(TF_i) \prod \Psi_j(TF_j). \quad \text{(C3)}$$

The absence of any one activating TF or the presence of any one repressing TF would both severely hinder transcription.

The binding site for an activating TF_A can be competitively occupied by a repressing TF_I; it has been suggested by von Dassow et al. (2000) that this can be represented by

$$\Phi(TF_A \cdot \Psi(TF_I)). \quad \text{(C4)}$$

As the exact binding sites of TFs are usually unclear and few instances of competitive binding have been confirmed experimentally, we treat all activating and repressing TFs equally using Eqn (B3). On the other hand, if a gene has multiple enhancers, its transcription can be turned on by the activation of any one enhancer. We therefore use the following equation to describe this OR condition

$$\frac{\sum \alpha_i E_i}{1+\sum \alpha_i E_i}. \quad \text{(C5)}$$

Here each E_i is of the form $\prod \Phi_i(TF_i) \prod \Psi_j(TF_j)$ and α_i is an E_i specific parameter indicating the strength of the enhancer. If a gene has a basal transcription rate, similarly it is controlled by a constant ON enhancer whose strength is *base*, plus the regulation by other activating or repressing TFs, in the form

$$\frac{base+\sum \alpha_i E_i}{1+base+\sum \alpha_i E_i}. \quad \text{(C6)}$$

D. Model equations

Equations contain the following classes of parameters:

parameter	meaning	parameter	meaning
taX_y	half-maximal activation coefficient (X transcriptionally activates the gene y)	*trX_y*	half-maximal inhibition coefficient (X transcriptionally inhibits the gene y)
paX_Y	half-maximal activation coefficient (X activates the protein Y)	*prX_Y*	half-maximal inhibition coefficient (X inhibits the protein Y)
k	protein binding rate	*c*	protein cleavage rate
base	base expression level	*H*	half-life
D	diffusion coefficient		

$$\frac{d\ dpp}{dt} = \frac{\ln 2}{H_{dpp}}\left(\left(\frac{CiA^{h20}}{ta_{CiA\_dpp}{}^{h20} + CiA^{h20}}\right) - dpp\right) \quad \text{(D1)}$$

$$\frac{d\,Dpp}{dt} = \frac{\ln 2}{H_{Dpp}}\left(dpp - Dpp\right) + D_{Dpp}\nabla^2 Dpp - k_{DppTkv} \cdot Dpp \cdot TkvF \quad \text{(D2)}$$

$$\frac{d\ tkv}{dt} = \frac{\ln 2}{H_{tkv}}\left(\frac{base_{tkv} + 10\left(\frac{MadA^{h10}}{ta_{Mad\_tkv}{}^{h10} + MadA^{h10}} \right)}{1 + base_{tkv} + 10\left(\frac{MadA^{h10}}{ta_{Mad\_tkv}{}^{h10} + MadA^{h10}} \right)} - tkv \right) \quad \text{(D3)}$$

$$\frac{d\ TkvF}{dt} = \frac{\ln 2}{H_{TkvF}}\left(tkv - TkvF\right) - k_{DppTkv} \cdot Dpp \cdot TkvF \quad \text{(D4)}$$

$$\frac{d\ TkvB}{dt} = \frac{\ln 2}{H_{TkvB}}\left(- TkvB\right) + k_{DppTkv} \cdot Dpp \cdot TkvF \quad \text{(D5)}$$

$$\frac{d\ mad}{dt} = \frac{\ln 2}{H_{mad}}\left(base_{mad} - mad\right) \quad \text{(D6)}$$

$$\frac{d\ Mad}{dt} = \frac{\ln 2}{H_{Mad}}\left(mad - Mad\right) - c_{Mad} \cdot Mad \cdot \left(\frac{TkvB^{h5}}{pa_{Tkv\_Mad}{}^{h5} + TkvB^{h5}} \right) \quad \text{(D7)}$$

$$\frac{d\ MadA}{dt} = \frac{\ln 2}{H_{MadA}}\left(-MadA\right) + c_{Mad} \cdot Mad \cdot \left(\frac{TkvB^{h5}}{pa_{Tkv\_Mad}{}^{h5} + TkvB^{h5}} \right) \quad \text{(D8)}$$

$$\begin{aligned} \frac{d\ hh}{dt} &= \frac{\ln 2}{H_{hh}}\left(1.0 - hh\right) \text{ in R2/R5, when Ato>ta}_{\text{Ato\_hh}} \text{ in R8} \\ &= \frac{\ln 2}{H_{hh}}\left(0.0 - hh\right) \text{ otherwise} \end{aligned} \quad \text{(D9)}$$

$$\frac{d\ Hh}{dt} = \frac{\ln 2}{H_{Hh}}\left(3.5 \cdot hh - Hh\right) + D_{Hh}\nabla^2 Hh - k_{HhPtc} \cdot Hh \cdot PtcF \quad \text{(D10)}$$

$$\frac{d\ ptc}{dt} = \frac{\ln 2}{H_{ptc}}\left(\frac{base_{ptc} + \left(\frac{tr_{CiR\_ptc}{}^{h10}}{tr_{CiR\_ptc}{}^{h10} + CiR^{h10}} \right)}{1 + base_{ptc} + \left(\frac{tr_{CiR\_ptc}{}^{h10}}{tr_{CiR\_ptc}{}^{h10} + CiR^{h10}} \right)} - ptc \right) \quad \text{(D11)}$$

$$\frac{d\ PtcF}{dt} = \frac{\ln 2}{H_{PtcF}}\left(ptc - PtcF\right) - k_{HhPtc} \cdot Hh \cdot PtcF \quad \text{(D12)}$$

$$\frac{d\ PtcB}{dt} = \frac{\ln 2}{H_{PtcB}}\left(- PtcB\right) + k_{HhPtc} \cdot Hh \cdot PtcF \quad \text{(D13)}$$

$$\frac{d\ ci}{dt} = \frac{\ln 2}{H_{ci}}\left(base_{ci} - ci\right) \quad \text{(D14)}$$

$$\begin{aligned} \frac{d\ CiA}{dt} = \frac{\ln 2}{H_{CiA}}\left(ci - CiA\right) &- c_{CiACiR} \cdot CiA \cdot \left(\frac{pr_{Ptc\_CiAR}{}^{h5 \cdot <Mad>}}{pr_{Ptc\_CiAR}{}^{h5 \cdot <Mad>} + PtcB^{h5 \cdot <Mad>}} \right) \\ &- c_{CiANil} \cdot CiA \cdot \left(\frac{HIB^{h5}}{pa_{HIB\_CiANil}{}^{h5} + HIB^{h5}} \right) \end{aligned} \quad \text{(D15)}$$

$$< Mad >= \frac{pr_{Mad\_CiAR} + MadA}{pr_{Mad\_CiAR}}$$

$$\frac{d\ CiR}{dt} = \frac{\ln 2}{H_{CiR}}\left(-CiR\right) + c_{CiACiR}\cdot CiA\cdot\left(\frac{pr_{Ptc\_CiAR}{}^{h5\cdot<Mad>}}{pr_{Ptc\_CiAR}{}^{h5\cdot<Mad>} + PtcB^{h5\cdot<Mad>}}\right) - c_{CiRNil}\cdot CiR\cdot\left(\frac{HIB^{h5}}{pa_{HIB\_CiRNil}{}^{h5} + HIB^{h5}}\right) \tag{D16}$$

$$\frac{d\ hib}{dt} = \frac{\ln 2}{H_{hib}}\left(\frac{10\left(\frac{CiA^{h10}}{ta_{CiA\_hib}{}^{h10} + CiA^{h10}}\right) + 10\left(\frac{HIB^{h10}}{ta_{HIB\_hib}{}^{h10} + HIB^{h10}}\right)}{1 + 10\left(\frac{CiA^{h10}}{ta_{CiA\_hib}{}^{h10} + CiA^{h10}}\right) + 10\left(\frac{HIB^{h10}}{ta_{HIB\_hib}{}^{h10} + HIB^{h10}}\right)} - hib\right) \tag{D17}$$

$$\frac{d\ HIB}{dt} = \frac{\ln 2}{H_{HIB}}\left(hib - HIB\right) \tag{D18}$$

$$\frac{d\ delta}{dt} = \frac{\ln 2}{H_{delta}}\left(\left(\frac{Ato^{h10}}{ta_{Ato\_delta}{}^{h10} + Ato^{h10}}\right)\left(\frac{tr_{NICD\_delta}{}^{h2}}{tr_{NICD\_delta}{}^{h2} + NICD^{h2}}\right) - delta\right) \tag{D19}$$

$$\frac{d\ Dl}{dt} = \frac{\ln 2}{H_{Dl}}\left(delta - Dl\right) - k_{DlN}\cdot Dl\cdot\sum_{i=1}^{4}\overline{NF}_i \tag{D20}$$

$$\frac{d\ sca}{dt} = \frac{\ln 2}{H_{sca}}\left(\left(\frac{Ato^{h10}}{ta_{Ato\_sca}{}^{h10} + Ato^{h10}}\right)\left(\frac{tr_{Ato\_sca}{}^{h10}}{tr_{Ato\_sca}{}^{h10} + Ato^{h10}}\right) - sca\right) \tag{D21}$$

$$\frac{dSca}{dt} = \frac{\ln 2}{H_{Sca}}\left(sca - Sca\right) + D_{Sca}\nabla^2 Sca - k_{ScaN}\cdot Sca\cdot NF - k_{AtoSca}\cdot Sca\cdot\frac{Ato}{pa_{Ato\_sca} + Ato} \tag{D22}$$

$$\frac{d\ notch}{dt} = \frac{\ln 2}{H_{notch}}\left(\frac{base_{notch} + 10\left(\frac{NICD^{h2}}{ta_{NICD\_notch}{}^{h2} + NICD^{h2}}\right)}{1 + base_{notch} + 10\left(\frac{NICD^{h2}}{ta_{NICD\_notch}{}^{h2} + NICD^{h2}}\right)} - notch\right) \tag{D23}$$

$$\frac{d\ NF}{dt} = \frac{\ln 2}{H_{NF}}\left(notch - NF\right) - k_{DlN}\cdot NF\cdot\sum_{i=1}^{4}\overline{Dl}_i - k_{ScaN}\cdot NF\cdot Sca \tag{D24}$$

$$\frac{d\ ND}{dt} = \frac{\ln 2}{H_{ND}}\left(-ND\right) + k_{DlN}\cdot NF\cdot\sum_{i=1}^{4}\overline{Dl}_i - c_N\cdot ND \tag{D25}$$

$$\frac{d\ NS}{dt} = \frac{\ln 2}{H_{NS}}\left(-NS\right) + k_{ScaN}\cdot Sca\cdot NF \tag{D26}$$

$$\frac{d\ NICD}{dt} = \frac{\ln 2}{H_{NICD}}\left(-NICD\right) + c_N\cdot ND \tag{D27}$$

$$\frac{d\ rho}{dt} = \frac{\ln 2}{H_{rho}}\left(\left(\frac{Ato^{h20}}{ta_{Ato\_rho}{}^{h20} + Ato^{h20}}\right) - rho\right) \tag{D28}$$

$$\frac{d\ Rho}{dt} = \frac{\ln 2}{H_{Rho}}\left(rho - Rho\right) \tag{D29}$$

$$\frac{d\ SpiR}{dt} = \frac{\ln 2}{H_{SpiR}}\left(base_{SpiR} - SpiR\right) - c_{SpiR}\cdot SpiR\cdot\left(\frac{Rho^{h10}}{pa_{Rho\_Spi}{}^{h10} + Rho^{h10}}\right) \tag{D30}$$

$$\frac{d\ SpiA}{dt}=\frac{\ln 2}{H_{SpiA}}\left(-SpiA\right)+D_{SpiA}\nabla^2 SpiA+c_{SpiR}\cdot SpiR\cdot\left(\frac{Rho^{h10}}{pa_{Rho\_Spi}{}^{h10}+Rho^{h10}}\right)$$
$$-k_{SpiEgfr}\cdot SpiA\cdot EgfrF \tag{D31}$$

$$\frac{d\ egfr}{dt}=\frac{\ln 2}{H_{egfr}}\left(base_{egfr}-egfr\right) \tag{D32}$$

$$\frac{d\ EgfrF}{dt}=\frac{\ln 2}{H_{EgfrF}}\left(egfr-EgfrF\right)-k_{SpiEgfr}\cdot SpiA\cdot EgfrF \tag{D33}$$

$$\frac{d\ EgfrB}{dt}=\frac{\ln 2}{H_{EgfrB}}\left(-EgfrB\right)+k_{SpiEgfr}\cdot SpiA\cdot EgfrF \tag{D34}$$

$$\frac{d\ MAPK}{dt}=\frac{\ln 2}{H_{MAPK}}\left(-MAPK\right)+c_{MAPK}\cdot\left(\frac{EgfrB^{h20}}{pa_{Egfr\_MAPK}{}^{h20}+EgfrB^{h20}}\right) \tag{D35}$$

$$\frac{d\ Inf}{dt}=\frac{\ln 2}{H_{Inf}}\left(-Inf\right)+D_{Inf}\nabla^2 Inf+c_{Inf}\cdot\left(\frac{MAPK^{h10}}{pa_{MAPK\_Inf}{}^{h10}+MAPK^{h10}}\right)-k_{InfXF}\cdot Inf\cdot XF \tag{D36}$$

$$\frac{d\ XF}{dt}=\frac{\ln 2}{H_{XF}}\left(base_{XF}-XF\right)-k_{InfXF}\cdot Inf\cdot XF \tag{D37}$$

$$\frac{d\ XB}{dt}=\frac{\ln 2}{H_{XB}}\left(-XB\right)+k_{InfXB}\cdot Inf\cdot XF \tag{D38}$$

$$\frac{d\ ro}{dt}=\frac{\ln 2}{H_{ro}}\left(\left(\frac{XB^{h10}}{ta_{XB\_ro}{}^{h10}+XB^{h10}}\right)\left(\frac{tr_{Rho\_ro}{}^{h10}}{tr_{Rho\_ro}{}^{h10}+Rho^{h10}}\right)-ro\right) \tag{D39}$$

$$\frac{d\ Ro}{dt}=\frac{\ln 2}{H_{Ro}}\left(ro-Ro\right) \tag{D40}$$

$$\frac{d\ ato}{dt}=\frac{\ln 2}{H_{ato}}\left(\frac{10<ato>+10\left(\frac{Ato^{h20}}{ta_{Ato\_ato}{}^{h20}+Ato^{h20}}\right)}{1+10<ato>+10\left(\frac{Ato^{h20}}{ta_{Ato\_ato}{}^{h20}+Ato^{h20}}\right)}-ato\right) \tag{D41}$$

$$<ato>=\left(\frac{tr_{NICD\_ato}{}^{h20}}{tr_{NICD\_ato}{}^{h20}+NICD^{h20}}\right)\left(\frac{tr_{Ro\_ato}{}^{h10}}{tr_{Ro\_ato}{}^{h10}+Ro^{h10}}\right)\left(\frac{CiA^{h10}}{ta_{CiA\_ato}{}^{h10}+CiA^{h10}}\right)$$

$$\frac{d\ Ato}{dt}=\frac{\ln 2}{H_{Ato}}\left(ato-Ato\right) \tag{D42}$$

Note:

1. $\overline{X}$: protein in neighbouring cells

2. h2=2, h5=5, h10=10, h20=20. In most equations the Hill coefficient is 10, which is largely reasonable because Ferrell, J. E. Jr. et al experimentally revealed more recently that the Hill coefficient for Stg activation by Cdk1 is about 11 (Trunnell, N. B., Poon, A. C., Kim, S. Y. & Ferrell, J. E. Jr. Ultrasensitivity in the regulation of Cdc25C by Cdk1. *Mol Cell* 2011, **41:**263–274). (2) Normally every biochemical reaction is nonlinear. When multiple biochemical reactions (or signaling events) are summarized into one reaction (one event), understandably, the nonlinearity of this unrealistic reaction should be very high, demanding a large Hill coefficient in the equation. MAPK activated by EgfrB is such a case; we compress the MAPK cascade into one reaction, which needs a large Hill coefficient.

3. $\nabla^2 U = \frac{U_{[1,0]} + U_{[-1,0]} + U_{[0,1]} + U_{[0,-1]} - 4 \cdot U_{[0,0]}}{L^2}$, L=0.2 is the length of the cell.

E. Biological basis of equations

D1(dpp): (i) *dpp* expression is activated by either the accumulation of CiA or the removal of CiR in a band of cells anterior to the furrow (Dominguez and Hafen, 1997; Pappu et al., 2003; Fu and Baker, 2003). (ii) Should *dpp* be activated by the removal of CiR, as Ci is degraded by HIB in cells posterior to the furrow, *dpp* would be expressed at high levels posterior to the furrow, which is against the observation (Pignoni and Zipursky, 1997; Chanut and Heberlein, 1997). We thus let *dpp* be activated by the accumulation of CiA.

D2(Dpp): Dpp is a diffusible ligand and binds to the receptor Tkv.

D3(tkv): Since UAS>>dpp (dpp overexpression) induces wide *hairy* expression, *tkv* should be widely expressed; since UAS>>tkv can induce uniformly high Hairy levels, its normal expression level should be able to be enhanced (Greenwood et al., 1999); and indeed the Tkv levels show somewhat difference in cells anterior and posterior to the furrow (Burke and Basler, 1996). We thus let tkv have a base level expression.

D4(TkvF): Tkv is a type I TGF-beta receptor required for all known Dpp activation.

D5(TkvB): Dpp binds to Tkv.

D6(mad): Since both UAS>>dpp and UAS>>tkv lead to high hairy expression, mad must be widely expressed. We thus let mad have a base level expression.

D7(Mad): Bound Tkv phosphorylates Mad into active form (Wiersdorff et al., 1996; Schmierer and Hill, 2007).

D8(MadA): Since the function of I-SMAD is not clear in this circumstance, only one active Mad (R-SMAD) is used, which is phosphorylated by the type I receptor upon ligand binding and functions as the effector of Dpp signalling (Wiersdorff et al., 1996; Schmierer and Hill, 2007).

D9(hh): hh is expressed first in R2/R5 cells and later in all developed photoreceptors except R8 (Rogers et al., 2005; Borod and Heberlein, 1998). Since the model does not simulate R2/R5 determination, we simply let hh be expressed in R2/R5 when the Ato level in R8 exceeds a threshold.

D10(Hh): (i) Hh is a diffusible ligand and binds to the receptor Ptc. (ii) Since hh is later expressed in all 7 differentiating photoreceptors (Rogers et al., 2005), here a factor 3.5 is used to compensate the hh levels.

D11(ptc): (i) ptc expression is low anterior to the furrow but high posterior to it, which is Hh-dependent (Heberlain et al., 1995; Strutt and Mlodzik, 1996; Dominguez and Hafen, 1997). This indicates a base expression level anterior to the furrow and an enhanced level by Hh signalling posterior to the furrow. If ptc is activated by high CiA (as is in the wing, see Alexandre et al., 1996; Muller and Basler, 2000), then the ubiquitinated CiA by HIB posterior to the furrow cannot explain the high Ptc levels; if it is activated by low CiR, then the high Ptc levels posterior to the furrow is a natural result of Ci ubiquitination. (ii) Since the response of ptc expression to CiA/CiR is context-specific rather than stereotyped (Muller and Balser, 2000), it could be activated by either the accumulation of CiA or the removal of CiR. (iii) As clones of ci-mutant cells differentiate normally in all respects, CiR could be more decisive in the eye (Fu and Baker, 2003). Taken together, we think ptc should be activated by the removal of CiR.

D12(PtcF): Hh binds to Ptc.

D13(PtcB): Hh binds to Ptc.

D14(ci): In the eye Ci is present in almost all cells (Dominguez, 1999; Pappu et al., 2003).

D15(CiA): (i)Around the furrow, Hh-bound Ptc stops the cleavage of CiA to CiR and causes the

accumulation of CiA (Chen et al., 1999; Ou et al., 2002; Fu and Baker, 2003); posterior to the furrow CiA is ubiquitinated by the Hh-induced MATH and BTB domain containing protein (HIB) (Zhang et al., 2006). (ii) Dpp signalling contributes to CiA accumulation, but not significantly, and it alone cannot activate ato (Greenwood et al., 1999; Baonza and Freeman 2001; Pappu et al., 2003). So it accelerates, but not directly causes, CiA accumulation.

D16(CiR): see D15(CiA).

D17(hib): (i) HIB expression is induced by high CiA posterior to the furrow (Zhang et al., 2006; Ou et al., 2007). (ii) Since CiA is ubiquitinated and constitutive HIB expression is observed in cells posterior to the furrow (Ou et al., 2002; Zhang et al., 2006), there should be a self positive feedback for HIB expression.

D18(HIB): No transformation/transport is known.

D19(delta): Dl expression is activated by Ato (Baker and Yu, 1998) and regulated by Notch signalling by lateral inhibition (Collier et al., 1996; Li and Baker, 2001) by the mechanism trans-inhibition (Bray et al 2006). In a negative feedback loop cells receiving the NICD down-regulate Dl expression, thus reducing their ability to signal back (Heitzler and Simpson 1991).

D20(Dl): Dl binds to N only on neighboring cells (Li and Baker, 2004; Le Borgne et al., 2005).

D21(sca): (i) sca expression is activated by Ato (Powell et al., 2001). (ii) Since Sca is not detected in more posterior ommatidia, its expression should be stopped in those cells (Li and Baker 2001). Without further data, we let it is stopped by Ato.

D22(Sca): (i) Sca is a diffusible N ligand (Mlodzik et al., 1990; Ellis et al., 1994; Powell et al., 2001), and it binds to N to stabilize N without producing downstream signal (Powell et al., 2001). (ii) Sca is later transported (probably by cell extension) to more posterior ommatidia (Chou et al., 2002). Without exact data, we assume this transport to be Ato activated.

D23(notch): N is widely expressed in all eye disk cells and its protein levels are uniform before the furrow (Baker and Yu, 1998). Its expression is regulated by Notch signalling by lateral inhibition (Collier et al., 1996; Li and Baker, 2001).

D24(NF): A cell is assumed to have four neighbors. N can be bound by Dl from the four direct neighbors and autonomous Dl/N binding is not allowed (Le Borgne et al., 2005). N is also competitively bound by Sca (Powell et al., 2001).

D25(ND): Dl-bound N is cleaved by Presenilin to produce NICD. When Presenilin is not concerned, its level is assumed at the steady state and the cleavage is a linear process (Raya et al., 2004).

D26(NS): Sca binds to N and stabilize N at the cell surface without producing downstream signal (Powell et al., 2001).

D27(NICD): see D25 (ND).

D28(rho): rho expression is activated by Ato (Wasserman et al., 2000; Baonza et al., 2001; Witt et al 2010).

D29(Rho): Rho functions as the modifier of Spitz.

D30(SpiR): SpiR is broadly expressed in cells, and the initial functionally inert SpiR is cleaved by Rho to become the soluble and active EGFR ligand SpiA (Wasserman et al., 2000; Shilo, 2003).

D31(SpiA): SpiA is a diffusible Egfr ligand (Freeman, 2002)(here it could be the proposed Spitz-2 , see Baonza et al., 2001).

D32(egfr): egfr is widely expressed in cells (Dominguez et al., 1998; Greenwood et al., 1999).

D33(EgfrF): see D32 (egfr)

D34(EgfrB): see D32 (egfr)

D35(MAPK): Egfr is responsible for all detectable MAPK activation in the furrow (Baonza et al.,

2001; Firth and Baker, 2007). EGFR activates MAPK signalling in proneural cells (Chen and Chien, 1999).

D36(Inf): (i) MAPK negatively and nonautonomously regulates ato expression (Chen and Chien, 1999), most likely by producing a diffusible inhibitory factor to activate ro (Spencer et al., 1998; Baonza et al., 2001). (ii) The inhibitory factor is unlikely Argos (Baonza et al., 2001; Yang and Baker, 2001; Klein et al., 2004), and as MAPK is held in cytoplasm for hours (Kumar et al., 2003), the inhibitory factor is likely produced by protein cleavage rather than by gene transcription.

D37(XF): The diffusible inhibitory factor should bind to some protein in target cells to activate ro expression. We assumed that the unknown receptor is widely expressed.

D38(XB): see D37 (XF)

D39(ro): (i) MAPK, via a hypothesized inhibitory factor, nonautonomously and positively regulates ro expression (Dominguez et al., 1998; Baonza et al., 2001). (ii) As Ro is found in most or all cells except R8 behind the furrow (Dokucu et al., 1996), we assume ro expression is repressed in R8 by Rho.

D40(Ro): see D40 (ro).

D41(ato): (i) ato activation is controlled by 5' and 3' enhancers (Sun et al., 1998). Hh signalling activates ato via the 3' enhancer and Ato self-activation is via the 5' enhancer (Dominguez, 1999; Chen et al., 1999). It is the 3' enhancer activity that is subject to the inhibition by Ro (Dominguez 1999; Baonza et al., 2001; Greenwood et al., 1999; Dokucu1996). (ii) ato expression is also regulated by Notch lateral inhibition (Baker and Yu, 1998). Since NICD represses ato in non-R8 cells with low Ato levels, it should be via the 3' enhancer.

D42(At0): see D41 (ato)

F. Initial and boundary conditions and their biological basis

Since we simulate the posterior-to-anterior moving of signalling in a 50x50 or 100x100 space of eye disc cells, at the dorsal and ventral (top and bottom) boundaries the periodic boundary condition is adopted, but at the anterior and posterior (left and right) boundaries the Neumann boundary condition is adopted. Since outside the anterior and posterior boundaries can be eye disc cells or other cells connecting to the eye disc, it is unreasonable to let diffusible molecules have fixed concentrations (the Dirichlet boundary condition, concentration=0.0 is a special case called sink condition) or fixed flux (the Neumann boundary condition, flux=0.0 is a special case called zero-flux condition) at the two boundaries. We assume that the diffusion of a diffusible molecule across cells at the boundaries is significant when the molecule's local concentration is high, but insignificant when the molecule's local concentration is low. Thus, the flux of a molecule is proportional (the factor is 0.5) to its known local concentration computed in the last round.

Initial values of molecules are as follows.

Molecule	Initial value	Molecule	Initial value	Molecule	Initial value
dpp	0.0	CiA	0.0	Rho	0.0
Dpp	0.0	CiR	baseci	SpiR	basespi
tkv	basetkv	hib	1.0 if in the column that generates R8s, 0.0 otherwise	SpiA	0.0
TkvF	basetkv	HIB	1.0 if in the	egfr	baseegfr

			column that generates R8s, 0.0 otherwise		
TkvB	0.0	delta	0.0	EgfrF	baseegfr
mad	basemad	Dl	0.0	EgfrB	0.0
Mad	basemad	sca	0.0	MAPK	0.0
MadA	0.0	Sca	0.0	Inf	0.0
hh	0.0	notch	basenotch	XF	baseXF
Hh	0.0	NF	basenotch	XB	0.0
ptc	baseptc	ND	0.0	ro	0.0
PtcF	baseptc	NS	0.0	Ro	0.0
PtcB	0.0	NICD	0.0	ato	0.4 if in the R8 precursors, 0.0 otherwise
ci	baseci	rho	0.0	Ato	0.4 if in the R8 precursors, 0.0 otherwise

G. Parameter values and their biological basis

Since the model describes the progression but not the initiation of the morphogenetic furrow and the progression is driven by newly determined R8s, a very simple initial condition is used. Initially, high Ato mRNA and protein levels exist in a column of R8 cells with an interval of 10 cells (Lee et al., 1996).

At the dorsal and ventral boundaries, since the ommatidia are surrounded by the same developing ommatidia, periodic boundary condition is used. At the anterior and posterior boundaries, since the developed ommatidia at the posterior end and the undetermined cells at the anterior end connect with other cells in the eye disck, the Robin boundary condition is used, which allows cross-boundary molecule diffusion and the diffusion is local concentration dependent.

Since most molecular concentrations and biochemical reactions are not quantitatively known, parameter values are determined upon the combined use of several means, including exploring constraints among parameters, tuning parameters to produce the observed phenotype, and following several general rules. These rules are:

(1) The half-life of mRNA molecules should be shorter than the half-life of proteins.
(2) Since a bound receptor is internalized into the cell and triggers downstream signalling, its half-life should be shorter than the unbound form.
(3) Similarly, the half-life of the active form of a protein should be shorter than the half-life of the inept form.
(4) If a pathway's response to an input signal is fast, then the activation of relevant genes and proteins is fast and the half-life of proteins is short.
(5) When without any information, we assume that the half-life of an mRNA is 10.0 and the half-life of a protein is 40.0 or 50.0.
(6) The binding affinity of a ligand to its receptor and the cleavage efficiency of a bound receptor is usually 1.0.

H_dpp=5.0 Rule (4).

H_Dpp=5.0	Rule (4) and tuned.
H_tkv=10.0	Rule (5).
H_TkvF=50.0	Rule (5).
H_TkvB=10.0	Rule (2).
H_mad=10.0	Rule (5).
H_Mad=40.0	Rule (5).
H_MadA=20.0	Rule (3).
H_hh=10.0	Rule (5).
H_Hh=25.0	Since Hh and Wnt pathways behave quite similarly in many respects (Lum and Beach, 2004) and Wg is a short-life ligand, the half-life of Hh may also be short.
H_ptc=10.0	Rule (5).
H_PtcF=50.0	Rule (5).
H_PtcB=10.5	Rule (2) and tuned. Although Hh diffuses into several ommatidia, *ato* is only activated in a band of cells with the width of one ommatidium. This demands either a large prPtc_CiAR to control the domain of CiA accumulation or a small H_PtcB to control the domain of bound Ptc.
H_ci=10.0	Rule (5).
H_CiA=40.0	Rule (5).
H_CiR=15.0	Tuned. As Ci75 is mainly in nuclei and Ci155 in the cytoplasm (Chen et al., 1999), this allows the effect of CiR removal comes quicker than the effect of CiA accumulation.
H_hib=10.0	Rule (5).
H_HIB=50.0	Rule (5).
H_delta=10.0	Rule (5).
H_Dl=40.0	Rule (5).
H_sca=5.0	Rule (4). *sca* is activated later than *delta* but its protein increases quick.
H_Sca=15.0	Rule (4). The experimentally measured half-life of Sca is very short (Ellis et al., 1994).
H_notch=10.0	Rule (5).
H_NF=50.0	Rule (5).
H_ND=30.0	Rule (2).
H_NS=30.0	Rule (2).
H_NICD=20.0	Tuned.
H_rho=10.0	Rule (5).
H_Rho=20.0	Rule (4). EGFR system responds quickly to activating signal (Spencer et al., 1998).
H_SpiR=50.0	Rule (5).
H_SpiA=20.0	Rule (3) and (4). EGFR system responds quickly to activating signal (Spencer et al., 1998).
H_egfr=10.0	Rule (5).
H_EgfrF=50.0	Rule (5).
H_EgfrB=15.0	Rule (2) and (4). EGFR system responds quickly to activating signal (Spencer et al., 1998).
H_MAPK=15.0	Rule (3) and (4). EGFR system responds quickly to activating signal (Spencer et al., 1998).
H_Inf=7.0	Rule (4). EGFR system responds quickly to activating signal (Spencer et al., 1998).
H_XF=50.0	Rule (5).
H_XB=15.0	Rule (2). EGFR system responds quickly to activating signal (Spencer et

	al., 1998) and *ato* repression by Ro first occurs in intervening cells.
H_ro=5.0	Tuned. *ato* repression by Ro first occurs in intervening cells.
H_Ro=7.0	Tuned. *ato* repression by Ro first occurs in intervening cells.
H_ato=10.0	Rule (5).
H_Ato=20.0	Tuned.
taMad_tkv=0.2	It should not be too large, as *tkv* expression can be significantly enhanced (Greenwood et al., 1999).
taCiA_dpp=0.06	It should be smaller than taCiA_ato, because, after activated by CiA, Dpp signalling should contribute to CiA accumulation.
taCiA_ato=0.2	It should be larger than taCiA_dpp, because Dpp and Hh signalling together contribute to CiA accumulation and ato activation.
taCiA_hib=0.5	CiA induces HIB expression at high concentration (Zhang et al., 2006)
trCiR_ptc=0.2	It should not be too small because, activated by Hh signalling, the feedback loop within the Hh pathway should gradually increase Ptc levels (Hebelain et al., 1995; Strutt and Mlodzik, 1996; Dominguez and Hafen, 1997).
taHIB_hib=0.1	It produces a reasonable timing of *HIB_Act_hib*.
trNICD_ato=0.003	It produces a timely repression of *ato* by Notch lateral inhibition.
trNICD_delta=0.001	Notch lateral inhibition has self-regulation during its contribution to Ato down regulation.
taNICD_notch=0.001	It is the same as trNICD_delta to allow balanced Notch lateral inhibition.
taXB_ro=0.0055	It should be small because a stable Ro distribution should be established in time to restrict *ato* expression activated by Hh signalling.
trRho_ro=0.1	*ro* should be timely repressed in R8 precursors (Docuku et al., 1996).
trRo_ato=0.07	It should be small to make Hh-induced *ato* expression spatially restricted timely.
taAto_delta=0.01	It should be small, significantly smaller than taAto_sca, because *Dl*, activated by Ato, is widely expressed in cells.
taAto_sca=0.1	It should be much larger than taAto_delta, because *sca*, activated by Ato, is observed only in proneural cells (Ellis et al., 1994; Jarman et al., 1995).
taAto_rho=0.35	It should be between taAto_sca and taAto_hh.
taAto_ato=0.45	It should be between taAto_sca and taAto_hh.
taAto_hh=0.75	It should be sufficiently larger than taAto_ato/taAto_rho, because the activation of *hh* by EGFR signalling in R2/R5 happens quite later (Rogers et al., 2005), probably due in part to the cytoplastic holding of dpERK (Kumar et al., 2003).
trAto_sca=0.8	It should be quite large because Sca is removed from mature R8 quite later (Chou et al., 2002).
prPtc_CiAR=0.0018	It should be very small, because the initial Ptc level in cells anterior to the furrow is very low (Strutt and Mlodzik, 1996) and its increase relies on the positive feedback between CiA and PtcB.
paTkv_Mad=0.15	It should not be too small. Since Dpp signalling does not significantly contribute to furrow movement (Greenwood et al., 1999; Pappu et al., 2003), we infer that MadA does not significantly contribute to CiA accumulation and the positive feedback between MadA and TkvB should not begin early.
prMad_CiAR=0.85	Since Dpp signalling does not significantly contribute to furrow movement (Greenwood et al., 1999; Pappu et al., 2003), we infer that MadA does not significantly contribute to CiA accumulation.

paRho_Spi=0.05	It should be small because EGFR signalling responds quickly to *ato* activation and Rho production is the limiting step of EGFR signalling (Spencer et al., 1998; Wasserman et al., 2000; Shilo, 2003).
paEGFR_MAPK=0.01	Similar to paRho_Spi.
paHIB_CiANil=0.2	Tuned. It produces a CiA distribution that works with the Ro distribution to induce the next column of R8 six cells ahead of the current R8.
paHIB_CiRNil=0.2	see paHIB_CiANil.
paAto_Sca=0.8	see trAto_sca
cMad=1.0	Rule (6).
cCiACiR=1.0	Rule (6).
cCiANil=0.5	Assumed.
cCiRNil=0.5	Assumed.
cMAPK=0.01	It should not be large because MAPK is produced only in proneural cells (Spencer et al., 1998; Chen and Chien, 1999).
cInf=0.1	It together with the tuned DifInf controls the domain of Inf.
cSpiRA=1.0	Rule (6).
cN=1.0	Rule (6).
kDppTkv=1.0	Rule (6).
kHhPtc=1.0	Rule (6).
kSpiEgfr=1.0	Rule (6).
kDlN=0.4	It should be small because the binding affinity between Dl/N should be much smaller than that between Sca/N (Powell et al., 2001).
kScaN=50.0	It should be much larger than kDlN (Powell et al., 2001).
kInfXF=1.0	Rule (6).
kAtoSca=1.0	Rule (6).
basetkv=0.3	It should not be large because *tkv* expression can be further enhanced by MadA (Burke and Basler, 1996), but it should be larger than baseptc.
basemad=0.9	mad is widely expressed and is not a limiting factor.
baseptc=0.04	Ptc levels are very low in cells anterior to the furrow (Strutt and Mlodzik, 1996).
baseci= 0.9	*ci* is widely and uniformly expressed in the eye disk (Dominguez, 1999; Pappu et al., 2003).
basespi=1.0	SpiR is widely expressed at high levels and Rho is the limiting factor of EGFR signalling (Wasserman et al., 2000).
baseegfr=0.9	*egfr* is widely expressed (Wasserman et al., 2000).
baseXF=0.9	Assumed.
basenotch=0.08	It should not be large because in proneural cells most N is hold by Sca (Powell et al., 2001).
DifDpp=0.05	It should not be large because Dpp distribution shows a clear band anterior to the furrow (Pignoni and Zipursky, 1997; Borod and Heberlain, 1998).
DifHh=0.7	It should be large because Hh can diffuse into several ommatidia (Dominguez, 1997).
DifSpiA=0.02	It should be small because the diffusion range of SpiA could be no more than two or three cells (Wasserman et al., 2000).
DifSca=0.028	It should be similar to DifSpiA because Sca distribution is observed in just 7-10 cells (Ellis et al., 1994).
DifInf=0.03	It should not be large because Inf diffusion is within an ommatidium.

Note: "assumed", in contrast to "tuned", means the arbitrarily chosen value works quite well and the value assigned to the parameter does not affect the system significantly.

H. Definition of signalling events

When the concentration of protein A reaches the half-maximal activation/repression coefficient in the Hill function describing how A nonlinearly activates/represses B (which can be a protein or a gene), A sends the message *activation* or *repression* to B, which is captured as *A_Act_B* or *A_Rep_B*. Upon experimental studies, ligand-receptor binding shows unclear or lesser nonlinearity than transcriptional activation and repression and protein activation and repression by phosphorylation. Lacking clear evidence whether ligand-receptor binding happens at particular concentrations, binding events may not be defined optimally (Supplementary Figure 3); this may influence not only the timing and duration, but also occurrence, of some binding events. For an event determined upon several other events, it is defined upon the logic relationship of the related events. Note that some events, such as *Ato_Act_hh_0_p1*, represent several steps of signaling occurred in or between cells. Defined events are captured in each and every cell during simulations (Supplementary Table 2). Definition and capture of events and numeric solution of equations were facilitated by a program (available upon request) developed for multi-cellular modeling (Zhu et al., 2005). The three kinds of events are defined as follows.

(1) *Events of molecular interaction or gene transcription controlled by threshold concentrations of proteins*

These events, including transcriptional activation/repression of genes and activating/repressive protein-protein interactions, are defined upon the half-maximal activation/repression coefficient in the Hill functions (Fig.1). For example, if *X>taX_y* then *X_Act_y* is ON; if *X>paX_Y* then *X_Act_Y* is ON.

(2) *Events of molecular interactions not controlled by threshold concentrations of proteins*

Binding of receptors by ligands is computed according to the mass-action rule. Since a bound receptor (for example, TkvB, PtcB, EGFRB and ND) leads to the production of a downstream signal molecule (for example, MadA, CiA, MAPK and NICD, respectively), which is regulated nonlinearly by a Hill function, we use the production of the downstream molecule to indicate ligand/receptor binding. We assume the signal transduction is fast, and use a small factor (=0.1), together with relevant parameters in Hill functions, to mark the occurrence of effective ligand/receptor binding. For example, if $k_{SpiEgfr} \cdot SpiA \cdot EgfrF > 0.1 \cdot pa_{Egfr\_MAPK}$, then SpiA sends a binding signal to EgfrF to indicate the occurrence of the event.

Nevertheless, there are two exceptions. First, since the Hh level is very low, we use $k_{HhPtc} \cdot Hh \cdot PtcF > 0.085 \cdot pr_{Ptc\_CiAR}$ to indicate the binding of Hh to PtcF. Second, since Sca binding to NF does not produce any target molecule, we use $k_{ScaN} \cdot Sca \cdot N > 0.1 \cdot base_{notch}$ to indicate the binding of Sca to NchF.

(3) *Derived events*

Derived events (mostly, the combinatorial control of gene expression) occur upon the occurrence of multiple simple events and are defined using the logic relationship between these simple events. For example, the expression of *ato* is regulated by four proteins at two enhancers of the *ato* gene (Fig.1) with the logic relationship $\left(\overline{NICD\_Rep\_ato} \cap \overline{Ro\_Rep\_ato} \cap CiA\_Act\_ato\right) \cup Ato\_Act\_ato$

Thus, the event ato_Exp_Ato is defined upon the events NICD_Rep_ato, Ro_Rep_ato, CiA_Act_ato and Ato_Act_ato.

Defined events are continuously captured in all cells during a simulation (Fig. 2). Note that definition and capture of events have no effect on computation of molecular concentration, and definition and capture of simple events do not affect each other.

I. Numerical solution

All differential equations were solved using the second-order Runge-Kutta method with adaptive time steps controlled by the two error thresholds *relerr* and *abserr*. For any molecule *U* satisfying $\frac{dU}{dt} = f(t,U)$, in the error control term $|err| < |U| \cdot relerr + abserr$, $err = \frac{k2 - k1}{2}$, $k1 = \Delta t \cdot f(t,U)$, $k2 = \Delta t \cdot f(t + \Delta t, U + k1)$, and *relerr*=*abserr*=0.0001. In simulations, as long as $|err| \geq |U| \cdot relerr + abserr$, the time step was halved.

3. Supplementary Figures and Tables

Supplementary Table 1 Molecules in the model.

dpp	Decapentaplegic mRNA	Sca	Scabrous protein
Dpp	Decapentaplegic protein	notch	Notch mRNA
tkv	Thick veins mRNA	NF	Free Notch protein
TkvF	Free Thick veins protein	ND	Delta-bound Notch
TkvB	Bound Thick veins protein	NS	Sca-bound Notch
mad	Mothers against dpp mRNA	NICD	Notch intracellular domain
Mad	Inactive Mothers against dpp protein	rho	Rhomboid mRNA
MadA	Active Mothers against dpp protein	Rho	Rhomboid protein
hh	Hedghog mRNA	SpiR	Inactive Spitz
Hh	Hedghog protein	SpiA	Active diffusible Spitz
ptc	Patched mRNA	egfr	EGFR mRNA
PtcF	Free Patched protein	EgfrF	Free EGFR protein
PtcB	Bound Patched protein	EgfrB	Bound EGFR protein
ci	Cubitus interruptus mRNA	MAPK	Phosphorylated (active) MAPK
CiA	Active Cubitus interruptus (Ci155)	Inf	Inhibitory factor
CiR	Repressive Cubitus interruptus (Ci75)	XF	The free unknown cell membrane protein that binds to Inf
hib	mRNA of the Hh-induced MATH and BTB domain containing protein	XB	The bound unknown cell membrane protein that binds to Inf
HIB	Hh-induced MATH and BTB domain containing protein	ro	Rough mRNA
delta	Delta mRNA	Ro	Rough protein
Dl	Delta protein	ato	Atonal mRNA
sca	Scabrous mRNA	Ato	Atonal protein

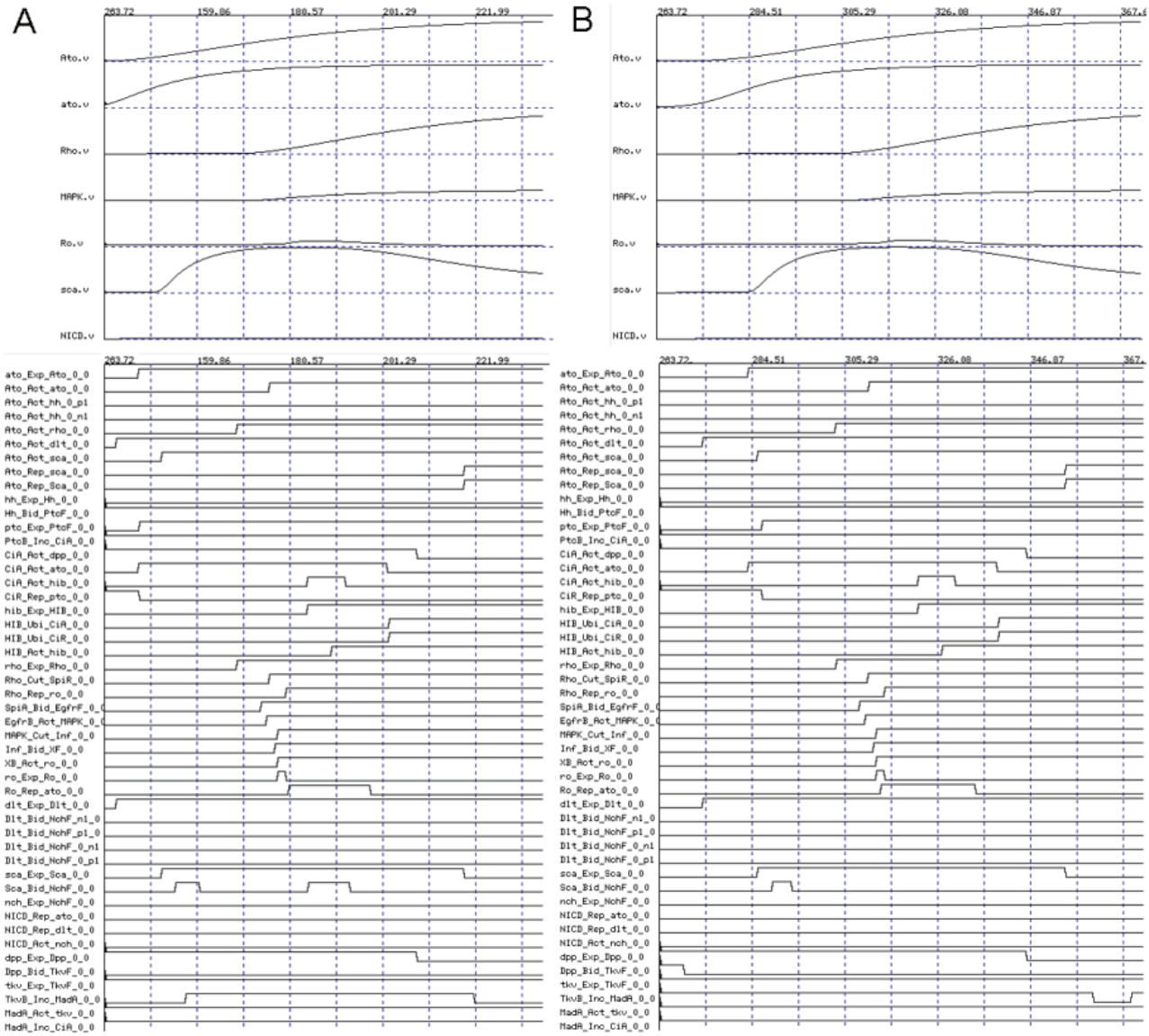
Molecules beginning with a capital letter are proteins, otherwise are mRNAs (and genes if in italic).

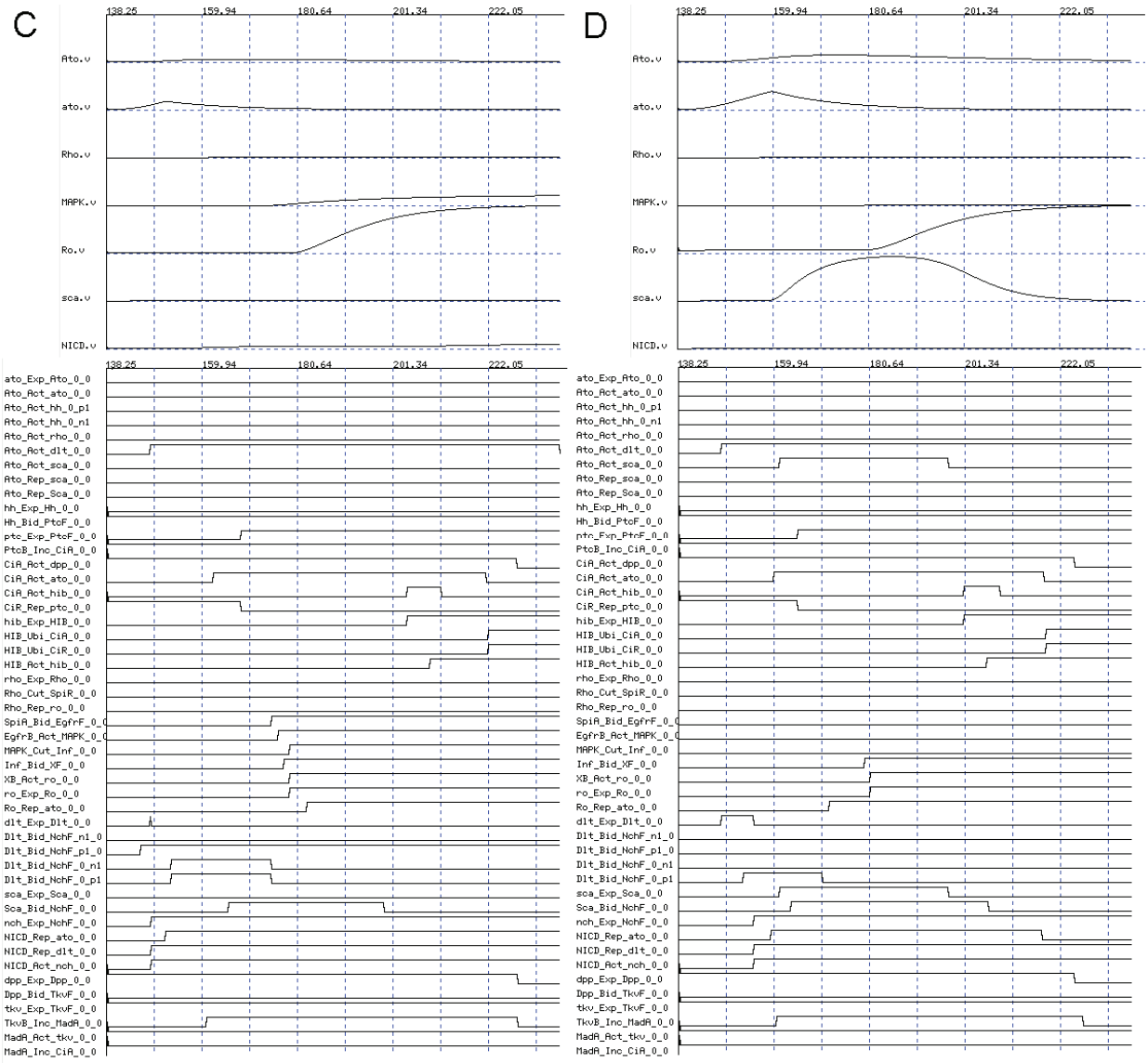
Supplementary Table 2 Defined and captured signaling events.

1.ato_Exp_Ato_0_0	13.PtcB_Inc_CiA_0_0	25.SpiA_Bid_EgfrF_0_0	37.sca_Exp_Sca_0_0
2.Ato_Act_ato_0_0	14.CiA_Act_dpp_0_0	26.EgfrB_Act_MAPK_0_0	38.Sca_Bid_NchF_0_0
3.Ato_Act_hh_0_p1	15.CiA_Act_ato_0_0	27.MAPK_Cut_Inf_0_0	39.nch_Exp_Nch_0_0
4.Ato_Act_hh_0_n1	16.CiA_Act_hib_0_0	28.Inf_Bid_XF_0_0	40.NICD_Rep_ato_0_0
5.Ato_Act_rho_0_0	17.CiR_Rep_ptc_0_0	29.XB_Act_ro_0_0	41.NICD_Rep_dlt_0_0
6.Ato_Act_delta_0_0	18.hib_Exp_HIB_0_0	30.ro_Exp_Ro_0_0	42.NICD_Act_nch _0_0
7.Ato_Act_sca_0_0	19.HIB_Ubi_CiA_0_0	31.Ro_Rep_ato_0_0	43.dpp_Exp_Dpp_0_0
8.Ato_Rep_sca_0_0	20.HIB_Ubi_CiR_0_0	32.dlt_Exp_Dlt_0_0	44.Dpp_Bid_TkvF_0_0
9.Ato_Rep_Sca_0_0	21.HIB_Act_hib_0_0	33.Dlt_Bid_NchF_n1_0	45.tkv_Exp_Tkv_0_0
10.hh_Exp_Hh_0_0	22.rho_Exp_Rho_0_0	34.Dlt_Bid_NchF_p1_0	46.TkvB_Inc_MadA_0_0
11.Hh_Bid_PtcF_0_0	23.Rho_Cut_SpiR_0_0	35.Dlt_Bid_NchF_0_n1	47.MadA_Act_tkv_0_0
12.ptc_Exp_Ptc_0_0	24.Rho_Rep_ro_0_0	36.Dlt_Bid_NchF_0_p1	48.MadA_Inc_CiA_0_0

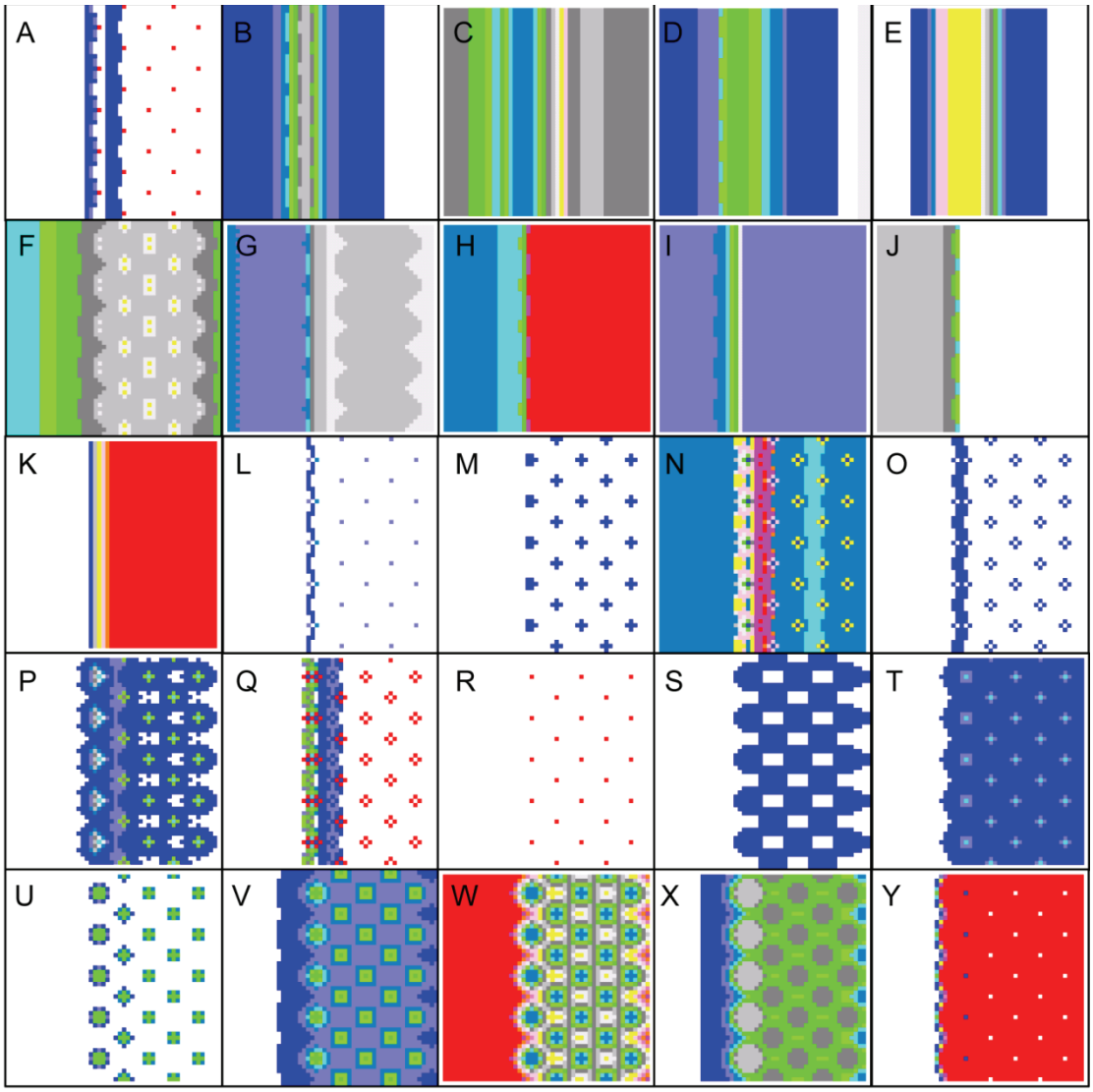
One message between two molecules, represented as *A_Message_B* that is defined in the model and captured as a message-passing from molecule A to molecule B in simulation,

indicates a signaling event. Messages can be arbitrarily defined. We defined Act (activation), Rep (repression), Inc (increase), Cut (cut/cleavage), Exp (expression), Bid (binding), and Ubi (ubiquitination). The relative cell address following *A_Message_B* indicates the position of the sender A. For example, the positions of the cells above and below the current cell (with the address [0,0]) are [0,+1] and [0,-1] and are represented as *_0_p1* and *_0_n1* in messages. In all following figures all events are numbered, and numbers without and with an underline indicate the start and stop of the events.





Supplementary Figure 1 Some molecular concentrations and signaling events in cells. (AB) In the R8 cell at [40,30] and [34,35]. **(C)** In the P cell at [39,30]. **(D)** In the I cell at [39,27]. (A) and (B) show round-to-round variations of signaling due to the changed occurrence, timing, and duration of some events. (A) and (C) reveal that *CiA_Act_ato* begins, and *CiR_Rep_ptc* stops, slightly earlier in R8 precursors than in other more anterior proneural cells. The rapid increase of Ato then causes *Dl*, *sca* and *rho* expression. Consequently, high Sca and Rho prevent the cell from Notch signaling and Ro expression, allowing Ato to increase unrepressed to reach the level of auto-regulation. In the I cell, since the cell is beyond the scope of SpiA diffused from the R8 precursor but within the scope of the inhibitory Inf from MAPK-active cells, there is *Ro_Rep_ato* but no *EgfrB_Act_MAPK*.



Supplementary Figure 2 Molecular concentrations in the 50x50 cell space at the time Ato=0.85 in the R cell at [22,25]. (A) Ato. (B) Dpp. (C) TkvF. (D) TkvB. (E) MadA. (F) Hh. (G) PtcF. (H) PtcB. (I) CiA. (J) CiR. (K) HIB. (L) Dl. (M) Sca. (N) NF. (O) ND. (P) NS. (Q) NICD. (R) Rho. (S) SpiA. (T) EgfrB. (U) MAPK. (V) Inf. (W) XF. (X) XB. (Y) Ro.

Supplementary Figures 3 and Supplementary Figure 4 show molecular concentrations at the time points when R8 precursors are selected in column 1 and column 4. The simulated results agree with reported expression profiles of these genes.

Ato (Fig. S3A, S4A)	Ato expression overlaps with the highest level of CiA (Dominguez, 1999) and is maintained in the differentiated R8. Ato-expressing clusters are initially bridged by 3-4 Ato-expressing cells and this bridge is then lost to refine Ato expression to separate proneural clusters and finally R8 (Dokucu et al., 1996). Compare with Fig. 4 in (Dokucu et al., 1996).
Dpp (Fig. S3B, S4B)	Dpp is expressed in the furrow where CiA levels are highest (Pignoni and Zipursky, 1997; Fu and Baker, 2003). Compare with Fig. 1 in (Pignoni and Zipursky, 1997).
Tkv (Fig. S3C, S4C)	Tkv is widely expressed in the eye disc, with expression enhanced in

	cells posterior to the furrow (Burke and Basler, 1996). Compare with Fig. 6 in (Burke and Basler, 1996).
TkvB (Fig. S3D, S4D)	
MadA (Fig. S3E, S4E)	
Hh (Fig. S3F, S4F)	Hh is expressed in R2/R5 cells and diffuses into cells anterior to the furrow. Compare with Fig2 in (Dominguez, 1997) and Fig2 in (Rogers et al., 2005).
Ptc (Fig. S3G, S4G)	Ptc is expressed weakly in cells anterior to the furrow and its expression is enhanced by Hh signalling in the furrow. Behind the furrow Ptc is expressed at a high level (Strut and Mlodzik 1996; Rangarajan et al., 2001). Compare with Fig. 2 in (Strut and Mlodzik, 1996) and Fig. 5 in (Rangarajan et al., 2001).
PtcB (Fig. S3H, S4H)	
CiA (Fig. S3I, S4I)	In the eye disc *ci* is expressed uniformly (Pappu et al., 2003). In cells anterior to the furrow, CiA is degraded into CiR and in cells posterior to the furrow it is ubiquitinated and degraded (Ou et al., 2007). Around the furrow bound Ptc results in the accumulation of CiA (Dominguez, 1999). Compare with Fig. 2 in (Dominguez, 1999) and Fig3 in (Ou et al., 2007).
CiR (Fig. S3J, S4J)	In cells anterior to the furrow CiA is degraded into CiR.
HIB (Fig. S3K, S4K)	HIB is induced by high levels of CiA posterior to the furrow. Compare with Fig. 1 in (Zhang et al., 2006).
Dl (Fig. S3L, S4L)	Activated by Ato, Dl is expressed uniformly but transiently in the furrow. The levels drop in intervening cells and increase later in differentiating ommatidia. Compare with Fig. 7 in (Fetchko et al., 2002) and Fig. 6 in (Baonza and Freeman, 2005).
Sca (Fig. S3M, S4M)	Sca is activated by Ato in proneural clusters (Powell et al., 2001). Compare with Fig. 6 in (Li and Baker, 2001) and Fig. 1 in (Ellis et al., 1994).
N (Fig. S3N, S4N)	Notch is widely expressed in all eye disc cells and is uniform anterior to the furrow (Baker and Yu, 1998). Compare with Fig. 7 in (Fetchko, et al., 2002) and Fig. 6 in (Baonza and Freeman, 2005).
ND (Fig. S3O, S4O)	Compare with Fig. 7 in (Fetchko et al., 2002).
NS (Fig. S3P, S4P)	
NICD (Fig. S3Q, S4Q)	Compare with E(spl) in Fig. 5 in (Dokucu et al., 1996) and in Fig. 6 in (Baker et al., 1996).
Rho (Fig. S3R, S4R)	Rho is activated by Ato in the furrow (Baonza et al., 2001) and its expression remains in R8 cells behind the furrow. Compare with Fig4 in (Spencer et al., 1998).
SpiA (Fig. S3S, S4S)	Functionally inert Spitz is cleaved by Rho to produce functional active diffusible Spitz (Wasserman et al., 2000; Shilo, 2003). Compare with Fig. 2 in (Tio and Moses, 1997).
EGFRB (Fig. S3T, S4T)	EGFR is expressed in all cells (Dominguez et al., 1998). Compare with Fig. 1 in (Dominguez et al., 1998).
MAPK (Fig. S3U, S4U)	MAPK is activated in evenly spaced proneural clusters in the furrow. Compare with Fig. 4 in (Spencer et al., 1998) and Fig. 1 in (Chen and Chien, 1999).
Inf (Fig. S3V, S4V)	This gives a prediction for the distribution of the hypothesized inhibitory factor (Baonza et al., 2001).
XF (Fig. S3W, S4W)	This gives a prediction for the expression of the membrane protein

	that binds to Inf.
XB (Fig. S3X, S4X)	This gives a prediction for the distribution of the Inf-bound membrane protein.
Ro (Fig. S3Y, S4Y)	The Ro protein is expressed first broadly within the furrow, non-overlapping with Ato, later specifically in R2/R5 followed by R3/R4 (Dokucu et al., 1996). Compare with Fig. 4 in (Dokucu et al., 1996).

4. Supplementary References

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