	Node	Logic functions
EGFR pathway	Egfr	input
	Rl	Egfr
	Pnt	Rl
	pGro	Rl | pGro
	Gro	!pGro
Wingless pathway	Wg	input
	Fz	Wg
	Arm	Fz
	Pan	Arm
Hedgehog pathway	Hh	input
	Ptc	!Hh
	Smo	!Ptc
	CiA	Smo & !en
	CiR	!Smo & !en
Markers	Dr	!vnd & !ind
	ind	!vnd & !Gro
	vnd	Pnt & !ind
	en	Pan
	hh	!CiR & en
	wg	CiA & !CiR
	gsb	!Ptc
	svp	ac | ind | vnd
	ac	Pan & !ind
	hkb	!Gro & !gsb
Neuroblast phenotypes	7-4	en & Dr & ac & svp
	7-1	en & vnd & ac & gsb & svp
	6-2	en & ind & gsb & svp
	5-6	wg & Dr & gsb
	5-3	wg & ind & gsb & svp
	5-2	wg & vnd & gsb & svp

Documentation of the logic functions

The EGFR Pathway

Egfr = input

Spi encodes ligand that activates Egfr. Aos functions as an inhibitor of the signaling triggered by Egfr by forming a clamp-like structure and sequestering Spi [9,10]. In the neuroectoderm model, we consider Egfr as an input to eliminate the redundancy originating from Spi inhibition by Aos. This way we can eliminate the redundant attractor where Egfr activation is not possible even though Spi is active.

Rl = Egfr
Rl encodes the mitogen activated protein (MAP) kinase, a core component of the RAS/MAPK pathway. Egfr activation induces RAS/MAPK pathway activation. Cells with a loss of function in Rl produce the same cell-death phenotype as seen in an EGF loss of function[11].

Pnt = Rl
Activated Rl phosphorylates and activates transcription factor Pnt[11,12].

pGro = Rl | pGro
Gro is phosphorylated by MAPK. Modification of Gro downregulates its repressor activity, causing derepression of pathway target genes. MAPK is no longer active after RTK signaling has been turned off, yet Gro remains stably phosphorylated and its activity attenuated, allowing for sustained RTK target gene expression. Phosphorylated Groucho is a nuclear and stable protein. This was captured in our model via self-loop of pGro and Gro inhibition[13].

Gro = ! pGro
Please refer to the function of pGro for the explanation of the Boolean update rule of Gro.

Wingless Pathway

Wg = input
Since the initial activations of *hh* and *wg* are independent of each other, Wg is provided as an input node[6]

Fz=Wg
The Wg signal is transduced across the membrane involving Frizzled proteins (such as Fz and DFz2) in the adjacent receiving cells[1].

Arm = Fz
Wg binding to Fz and Arrow brings them together, thereby recruiting Dsh to the membrane. When the destruction complex is inactivated by receptor/Dsh activity, Arm/beta-catenin translocates to the nucleus where it binds the N-terminus of Pan, displacing the Groucho co-repressor and recruiting activators to drive target gene expression. The recruitment of Dsh to the membrane is not included in the model. We considered the main event that Wg binding to Fz activates Arm translocation to the nucleus where it binds to the N-terminus of Pan, thereby recruiting activators to drive target gene expression[7,8].

Pan = Arm
Please refer to the function of Arm.

Hedgehog Pathway

Hh = input
Hh is an input node as it is a morphogen and can activate Hh signal transduction pathway. **2.**

Ptc=!Hh
Secreted Hh interacts with its receptor Ptc, thus relieving the repression of Ptc on Smo[1,2].

Smo=!Ptc
Please refer to the function of Ptc. In the absence of Hh ligand, repression of Smo allows Drosophila Protein Kinase A (PKA) phosphorylation of Ci on several sites, and these phosphorylation events are required for the cleavage of Ci into Ci^{R} [3].

Ci^{A}=Smo&!en
Ci is a cytoplasmic protein with no known function in this form. It can be cleaved to generate Ci^{R}, a 75 kD transcriptional repressor (henceforth Ci^{R}) or full-length Ci, a 155 kD transcriptional activator (Ci^{A}). Smo is freed of the inhibitory effects of Ptc, Smo signals through unknown mechanisms to the Fu/Cos2/Ci complex, causing hyperphosphorylation of Fu and Cos2. This results in the complex loosening its hold on microtubules and leads to the stabilization of Ci^{A}. Stabilized Ci^{A} can then travel to the nucleus and functions as a transcriptional activator, upregulating transcription of Hh target genes[4,5].

Ci^{R} =!Smo&!en
Pka phosphorylates Ci on several sites and these phosphorylation events are required for the cleavage of Ci into Ci^{R}. Ci is repressed by *en*. Since the exact mechanism leading to the generation of the different forms of Ci is not fully known, these reactions were omitted from the model. Ci repression by *en* was introduced into the model via two inhibitory edges to both forms of Ci [4,5]**.**

Markers

Dr = !vnd & !ind
Please refer to reaction 21 for the explanation of the Boolean update rule of *Dr*.

ind = !vnd & !Gro
Vnd represses *ind* and *Dr* in the ventral neuroectoderm, and *ind* represses *Dr* in the intermediate neuroectoderm. Gro is a nuclear repressor and represses *ind* transcription[15–17].

vnd = Pnt & !ind
EGF signaling and Pnt either directly or indirectly maintain the expression of several genes in the neurogenic ectoderm including *ind* and *vnd*, which encode regulatory proteins that pattern the future ventral nerve cord. EGF signaling maintains the expression of Pnt transcription factor, which, in turn, sustains the expression of *vnd* (previously activated by Dorsal and Twi), Rho, and Vn[13– 15]

en = Pan
Please refer to reaction 9 for the explanation of the Boolean update rule of *en*.

wg= CiA & !CiR
Ci^R represses *wg*, *ptc*, and *hh* transcription whereas Ci^A induces transcription of *ptc* and *wg*[4,5].

hh = en & ! Ci^R
One of the functions of *en* is to maintain *hh* expression. Ci^R, a 75 kD transcriptional repressor moves to the nucleus and represses *hh*[1].

gsb =! Ptc
Ptc represses the expression of *gsb*'[14].

svp = ac | ind | vnd
Vnd controls activation of two other genes, *hkb* and *svp*. The row 5 NB5-2 expresses svp-lacZ and divides asymmetrically multiple times. In gsb- embryos, both row 3 and row 5 medial neuroblasts express the row 3 markers *ftz* and *odd*, do not express the row 5 marker svp-lacZ, and divide nearly symmetrically[27].

ac = Pan & !ind
Ac is repressed in the neurectoderm and neuroblasts by *ind*. Arm and *pan* regulate the transcriptional activation of numerous target genes such as *ac*[18].Since *arm* is a transcriptional activator of *pan*, *arm* indirectly involved in the activation of *ac*. Therefore, in the neuroectoderm model, *pan* activates *ac*. In addition to activation of AS-C[19], *vnd* also controls activation of two other genes, *hkb* and *svp*, in the proneural clusters[20].

hkb = !Gro & !gsb
Both Wg and Hh activate *hkb* expression in the neuroectoderm and NBs, and multiple repressors act to restrict its expression. *En* is expressed in neuroectodermal rows 6/7 and partially represses *hkb* expression in those rows[21]. *Vnd* controls activation of *hkb*[20].

Neuroblast fates

7-4 = en & Dr & ac & svp

In Drosophila NB7-4 expresses *en* and *ac* as it delaminates[22–24].

7-1 = en & vnd & gsb & ac & svp

NB7-1 expresses *gsb*, *en*, *vnd*, and *ac* as it forms during S1[19,25–27].

6-2 =en & ind & gsb & svp

At S2, NB 6-2 expresses en and ind, svp. Gsb expression is detected by S3[24,25,29]

5-6 = wg & Dr & gsb

At S1, NB5-6 expresses Dr, *wg* and *gsb-d* [23,25,27,28]

5-3 = wg & ind & gsb & svp

At S1 NB 5-3 expresses *wg* [25,28], *ind*[29], and *gsb-d*[23,25,27]. At S2 it adds svp expression[25] .

5-2 = wg & vnd & gsb & svp

NB 5-2 expresses *svp-lacZ*, *gsb-d*, *vnd*[26,30] and *wg* as it forms[25,27,31] .

Neuroectodermal (NE) fates

Neuroectoderm patterns by the expression of three genes- vnd, ind, Dr in the neuroectodermal cells. (We expect at least three attractors with the activity of Dr, ind and vnd separately.) Other transcriptional factors expressed by these cells but not spatially restricted include *hkb* and svp[20]. Egfr and pGro input combinations reflect the neuroectodermal cell states as it leads to the activation of transcriptional factors: vnd, ind, Dr. The EGFR pathway is sufficient to generate multiple neuroectodermal cell fates by both internal (via phosphorylation of Gro) or external signals (via receptor activation).

Attractors associated with the NE fate include the following transcription factor activities:

Dr
ind, svp, hkb
vnd, svp, hkb

Non-physiological (NP) fates

The model revealed four attractors representing non-physiological states, characterized by combinations of marker expressions not observed in neuroblasts and neuroectoderm under normal conditions. Among these non-physiological attractors, one stands out with the predicted active genes Dr, en, svp, ac, and gsb. Typically, these gene expressions are not observed in neuroblasts, except for the exclusion of gsb expression in NB7-4.

Attractors associated with the NP fate include the following transcription factor activities:

Dr, en, gsb, svp, ac
ind, en, svp, hkb
vnd, en, svp, ac, hkb

In addition, any other combinations of marker gene activities that have not been defined as NE or cell type (e.g.7-1).

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