|  |  |  |
| --- | --- | --- |
| **Gene symbol** | **Gene ID** | **Gene name** |
| hh | FBgn0004644 | hedgehog |
| ptc | FBgn0003892 | patched |
| smo | FBgn0003444 | smoothened |
| ci | FBgn0004859 | cubitus interruptus |
| Pka-C2 | FBgn0000274 | Protein kinase, cAMP-dependent, catalytic subunit 2 |
| gsb | FBgn0001148 | gooseberry |
| wg | FBgn0284084 | wingless |
| fz | FBgn0001085 | frizzled |
| pan | FBgn0085432 | pangolin |
| arm | FBgn0000117 | armadillo |
| en | FBgn0000577 | engrailed |
| Egfr | FBgn0003731 | Epidermal growth factor receptor |
| spi | FBgn0005672 | spitz |
| aos | FBgn0004569 | argos |
| rl | FBgn0003256 | rolled |
| pnt | FBgn0003118 | pointed |
| gro | FBgn0001139 | groucho |
| ind | FBgn0025776 | intermediate neuroblasts defective |
| vnd | FBgn0261930 | ventral nervous system defective |
| Dr | FBgn0000492 | Drop, muscle segment homeobox |
| ac | FBgn0000022 | achaete |
| hkb | FBgn0000022 | huckebein |
| svp | FBgn0003651 | seven up |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Node** | | **Logic functions** |
| Hedgehog  Pathway | 1. | Hh | input |
| 2. | Ptc | !Hh |
| 3. | Smo | !Ptc |
| 4. | Pka | !Smo |
| 5. | CiA | Smo & !en |
| 6. | CiR | Pka & !en |
| Wingless  Pathway | 7. | Wg | input |
| 8. | Fz | Wg |
| 9. | Arm | Fz |
| 10. | Pan | Arm |
| Egfr  Pathway | 11. | Egfr | input |
| 12. | Rl | Egfr |
| 13. | Pnt | Rl |
| 14. | pGro | Rl | pGro |
| 15. | Gro | !pGro |
| Segment  Polarity  Genes | 16. | wg | CiA & !CiR |
| 17. | en | Pan |
| 18. | hh | en & !CiR |
| Columnar  Genes | 19. | vnd | Pnt & !ind |
| 20. | ind | !vnd & !Gro |
| 21. | Dr | !vnd & !ind |
| Other Marker Genes | 22. | svp | vnd | gsb |
| 24. | ac | !ind & (vnd | Arm) |
| 25. | hkb | (wg | hh | vnd) & !en |
| Neuroblast Fates | 26. | 7-4 | en & ac |
| 27. | 7-1 | en & vnd & gsb & ac |
| 28. | 5-6 | wg & gsb |
| 29. | 5-3 | wg & ind & gsb |
| 30. | 5-2 | wg & vnd & gsb & svp |

**Documentation of the logic functions**

**Hedgehog Pathway**

1. **Hh = input**

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

1. **Ptc = !Hh**

Secreted Hh interacts with its receptor Ptc, thus relieving the repression of Ptc on Smo1,2.

1. **Smo = !Ptc**

Please refer to reaction 5 for the explanation of the Boolean update rule of Smo.

1. **Pka = ! Smo**

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 CiR 3.

1. **CiA = Smo & ! En**

Ci is a cytoplasmic protein with no known function in this form. It can be cleaved to generate CiR, a 75 kD transcriptional repressor (henceforth CiR) or full-length Ci, a 155 kD transcriptional activator (CiA). 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 to loosen its hold on microtubules and leads to the stabilization of CiA. Stabilized CiA can then travel to the nucleus and functions as a transcriptional activator, upregulating transcription of Hh target genes4,5.

1. **CiR = Pka & ! En**

Pka phosphorylates Ci on several sites and these phosphorylation events are required for the cleavage of Ci into CiR. 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**.**

**Wingless Pathway**

1. **Wg = input**
2. **Fz = Wg**

The Wg signal is transduced across the membrane involving Frizzled proteins (such as Fz and DFz2) in the adjacent receiving cells1.

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 Tcf (also known as dTCF or 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. Wg protein that is transcribed and secreted from an anterior row of cells maintains the expression of a transcription factor, En, in adjoining, posterior cells 6,7.

1. **Pan = Arm**

Please refer to reaction [4](#_top) for the explanation of the Boolean update rule of Pan.

**EGFR Pathway**

1. **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 8,9. 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.

1. **Rl = Egfr**

Rl encodes the mitogen activated protein (MAP) kinase, a core component of the RAS/MAPK pathway. Egfr activation induces RAS/MAPK pathway. Cells with a loss of function in Rl produce the same cell-death phenotype as seen in an EGF loss of function10.

1. **Pnt = Rl**

Activated Rl phosphorylates and activates transcription factors such as Pnt10,11.

1. **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 inhibition12.

1. **Gro = !pGro**

Please refer to reaction 14 for the explanation of the Boolean update rule of Gro.

**Segment Polarity Genes**

1. **wg = CiA & ! CiR**

CiR represses wg, ptc, and hh transcription whereas CiA induces transcription of ptc and  wg4,5.

1. **gsb = wg & !Ptc**

Ptc represses expression of gsb by repressing wg, an activator of gsb1. The main function of gsb, which encodes a transcription factor containing a paired-domain and a prd-type homeodomain, is the maintenance of wg expression by a wg-gsb autoregulatory loop after 6 h of development13.

1. **en = Pan**

Please refer to reaction [9](#_top) for the explanation of the Boolean update rule of en.

1. **hh = en & !CiR**

One of the functions of En is to maintain *hh* expression. CiR, a 75 kD transcriptional repressor moves to the nucleus and represses *hh*1.

**Columnar genes**

1. **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 Vn13– 15.

1. **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 transcription14–16.

1. **Dr = !Vnd & !Ind**

Please refer to reaction 18 for the explanation of the Boolean update rule of Dr.

**Other markers**

1. **Svp = vnd | gsb**

Vnd controls activation of two other genes, hkb and svp. The row 5 neuroblast 5-2 (arrowhead) expresses svp-lacZ (c) and divides asymmetrically (inset, c) multiple times. In gsb- embryos, both row 3 (arrow) and row 5 (arrowhead) medial neuroblasts express the row 3 markers ftz and odd (d, e), do not express the row 5 marker svp-lacZ (f), and divide nearly symmetrically (Skeath, J., Zhang, Y., Holmgren, R. et al

1. **Ac = !ind & (vnd | Arm)**

Achaete (Ac) is repressed in the neurectoderm and neuroblasts by ind. Arm and pan regulate the transcriptional activation of numerous target genes such as achaete (ac)17. In addition to activation of AS-C18, vnd also controls activation of two other genes, hkb and svp, in the proneural clusters19.

1. **Hkb=(wg | hh | vnd) & !en**

Both Wg and Hh activate hkb expression in the neuroectoderm and NBs, and multiple re- pressors act to restrict its expression. En is expressed in neuroectodermal rows 6/7 and partially represses hkb expression in those rows20. Vnd controls activation of hkb19.

**Neuroblast fates**

1. **7-4 = en & ac**

In Drosophila NB 7-4 expresses en and ac as it delaminates21–23.

1. **7-1 = en & vnd & gsb & ac**

NB 7-1 expresses *gsb-d*, *en*, *vnd*, and *ac* as it forms during S118,24–26.

1. **5-6 = wg & gsb**

At S1, NB 5-6 expresses wg and gsb-d 22,24,26,27

1. **5-3 = wg & ind & gsb**

At S1 NB 5-3 expresses wg 24,27, ind28, and gsb-d22,24,26.

1. **5-2 = wg & vnd & gsb & svp**

NB 5-2 expresses svp-lacZ, gsb-d, vnd25,29 and wg as it forms24,26,30 .

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