**Outlines of Steppables from palate\_steppables.py**

Behaviors (Pseudo-code)

1. Each cell carries information
   1. On lineage (generation, parent and parentClusterId; for informational purposes only; nothing in model depends on these values)
   2. 3 flags:
      1. needsClusterReassignmentFlag (used to make sure clusters are handled correctly during mitosis)
      2. apoptosisFlag (true if cell has committed to apoptosis)
      3. mitosisFlag (true if cell is ready to divide)
   3. 3 timers:
      1. ectoDivision (counts down toward zero – for EPI and PERI cells only; initiated with a random integer between 1 and 101)
      2. apoptosis (time since commitment to apoptotic pathway)
      3. mitosis (time since last cell division; only used for readouts; nothing in model depends on this)
   4. On EGFR level (set to 1 for all EPI and PERI cell types and zero otherwise; this is only present as a check on cell type assignments; nothing in model depends on this)
2. Setting the initial cell geometry (SetInitial2DGeometry)
   1. Make a 1-pixel wide Wall around entire simulation (keeps cells from sticking to sides of simulation box)
   2. Specify region dimensions & locations for each palate shelf
      1. Mesench = part of a circle
         1. {x0, y0} = {-3/64 YY = -18.75, ½ YY = 200} and {XX + 3/64 YY = 218.75, ½ YY = 200}; centered vertically, but centers are just off-lattice horizontally
         2. radii = 3/32 YY = 37.5
      2. Epi = part of circular shell surrounding Mesench
         1. Shell thickness = 5
      3. Peri = part of circular shell surrounding Mesench+Epi
         1. Shell thickness = 4
   3. Specify cells in each region
      1. Select N random cell seed locations with N = {35, 20, 10) for Mesench, Epi and Peri regions respectively
      2. Assign Mesench cell seeds to be oral or nasal type based on whether location is above or below midline of shelf
      3. Assign Epi and Peri cell seeds to be oral, medial or nasal based on whether seed location is 30 or more degree above midline, between +/- 30 degrees, or more negative than -30 degrees below midline
      4. Assign each lattice site in a region to cell ID associated with closest seed location in that region
   4. Above parameters yield average cell volumes as shown at end of this file (Mesench ~20-25, Epi ~ 20, Peri ~ 40)
   5. Note that initial specification defines no “cells” of type Matrix, BM, Mesench\_EMT, or Peri\_\*a; these will come later from cell behaviors
3. Set volume at which mitosis occurs (MITOSIS\_VOLUME\_LIST)
   1. Mesench cells:
      1. 2\*(avg. initial volume of Mesench type with greatest initial volume)
      2. {makes sure mitosis volume isn’t zero if no such cells are initially specified}
   2. Epi cells:
      1. f\*(avg. initial volume of Epi type with greatest initial volume)
         1. f = 2 for EPI\_N and EPI\_O
         2. f = 3 for EPI\_ME, which makes it a little more difficult for them to undergo mitosis
   3. Peri cells:
      1. f\*(avg. initial volume of Peri type with greatest initial volume)
         1. f = 2 for PERI\_N and PERI\_O
         2. f = 3 for PERI\_ME, which makes it a little more difficult for them to undergo mitosis
4. Set initial cell shape parameters
   1. Volume and surface area terms in pseudo-energy
      1. target volume and surface area set for each cell to equal initial volume and surface area
      2. can be changed so targets equal averages for each cell type, but this was not used in sims presented here
      3. For Mesench cells, option to set target volume to a random number between 5% and 95% of the Mesench mitosis volume
         1. this was used
         2. target surface area then set to 4\*Sqrt(target\_volume) – biases toward cuboidal cells
      4. lambda\_volume = 0.5, lambda\_surface = 0.1
         1. except for PERI cells, where lambda\_volume = 2.0
   2. Length terms in pseudo-energy
      1. Only set for PERI cells
      2. Lamba\_length = 20\*lambda\_surface = 2.0
      3. Target length = 0.3\*(target volume)
5. Behaviors called every N Monte Carlo steps (N = [self.frequency] = 10)
   1. Apoptosis
      1. If a cell has already committed to apoptosis (apoptosisFlag=True for any compartment in cell), then …
         1. Reduce target volume by (N/ apoptosisTimeConstant)\*targetVolume
            1. apoptosisTimeConstant = 120
            2. leads to exponential decay of targetVolume
         2. Set target surface area = 4\*Sqrt(targetVolume)
         3. Remove length term (lamba\_length -> 0) if cell had one
      2. Decide whether cell enters apoptosis (probabilistically)
         1. Not allowed during first 200 MCS
         2. If cell not in contact with any other cell or matrix . . .
            1. Increase prob by 0.1 per MCS (i.e., a 10% chance per cell per MCS)
         3. Else if cell is PERI\_\*a type
            1. Increase prob by Hill[TGFb3, 0.0001, AC50=70]
            2. Decrease prob by Hill[EGF, 0.0001\*ahrDrivenEGFRFoldChange, AC50=70]
            3. If not in contact with Medium:

Increase prob by Hill[TGFb3, 0.01, AC50=70]

Decrease prob by Hill[EGF, 0.01\*ahrDrivenEGFRFoldChange, AC50=70]

* + - * 1. If not in contact with basal PERI compartment:

Increase prob by Hill[TGFb3, 0.01, AC50=70]

Decrease prob by Hill[EGF, 0.01\*ahrDrivenEGFRFoldChange, AC50=70]

* + - * 1. 
      1. Else if cell is PERI\_\* type
         1. Increase prob by Hill[TGFb3, 0.0001, AC50=70]
         2. Decrease prob by Hill[EGF, 0.0001\*ahrDrivenEGFRFoldChange, AC50=70]
         3. If not in contact with either Medium or PERI\_\*a:

Increase prob by Hill[TGFb3, 0.01, AC50=70]

Decrease prob by Hill[EGF, 0.01\*ahrDrivenEGFRFoldChange, AC50=70]

* + - * 1. Else if not in contact with either PERI\_\* or PERI\_\*a:

Increase prob by Hill[TGFb3, 0.01, AC50=70]

Decrease prob by Hill[EGF, 0.01\*ahrDrivenEGFRFoldChange, AC50=70]

* + - * 1. 
      1. Else if cell is EPI\_\* type
         1. Increase prob by Hill[TGFb3, 0.00002, AC50=70]
         2. Decrease prob by Hill[EGF, 0.00002\*ahrDrivenEGFRFoldChange, AC50=70]
         3. If not in contact with either Medium or any PERI:

Increase prob by Hill[TGFb3, 0.002, AC50=70]

Decrease prob by Hill[EGF, 0.002\*ahrDrivenEGFRFoldChange, AC50=70]

* + - * 1. 
      1. Increase prob by a factor of 5 (not sure why this came in late)
      2. Set prob after a step of N MCS based on prob per MCS:
         1. probN = 1 – (1 - prob)^N
      3. use random number generator to decide whether cell commits to apoptosis
  1. Mitosis
     1. Decide whether cell will divide
        1. Limited to actual cells (excludes Matrix and BM “cells”)
        2. Requires entire cell volume > cell type’s mitosis trigger volume
        3. Could require minimum aspect ratio, but not used
        4. Requires for Epi and Peri cells that a certain number of MCS have passed since last mitosis (countdown timer ectoDivision <=0)
           1. timer initiated at random value between 1 and 101 for cells of type EPI or PERI;
           2. reset to 50 for EPI and PERI cells after a cell division
           3. timer always 0 for other cell types
     2. Execute cell divisions
        1. For Mesench types, division plane is random
        2. For EPI and PERI types, division plane is along minor axis (i.e., elongated cells become shorter after division)
        3. Code to make sure all compartments of child cell get same clusterID
        4. Target volume split between child and parent cell with child fraction set to a random number between .465 and .535
        5. Target surface area set based on target volume
           1. For Mesench and Epi:

target surface = 4\*Sqrt(target volume)

* + - * 1. For basal Peri:

target surface = 0.7\*(target volume)

* + - * 1. For apical Peri:

target surface = 1.0\*(target volume)

* + - 1. Target length of Peri cells set based on target volume
         1. For basal Peri:

Target length = 0.3\*(target volume)

* + - * 1. For apical Peri:

Target length = 0.5\*(target volume)

* + - 1. For Epi and Peri cells, set ectoDivision timer back to 50 (counts down to zero)
      2. Increase generation counter by 1 for parent cell
      3. Reset mitosis timers back to 0 (counts up)
  1. Motility
     1. Motility of PERI cells varies with time via changes to fluctuationAmplitude parameter for just those cells
     2. Changes begin only after 300 MCS
        1. New base fluctuationAmplitude is 2 (half that of other cells)
        2. Increase fluctuationAmplitude by Hill[TGFb3, 8, AC50=70]
        3. Decrease fluctuationAmplitude by Hill[EGF, 8\*ahrDrivenEGFRFoldChange, AC50=70]
        4. Don’t ever let fluctuationAmplitude go below zero
        5. Summary equation is
     3. There is a variable for when to increase PERI cell motility (set to 5010 MCS), but it is not used (effect is commented out). Double-checked and this is not actually used in the model
  2. Differentiation
     1. Only allowed after 200 MCS
     2. Only occurs for EPI cells
     3. Decide whether an EPI cell undergoes an epithelial-to-mesenchymal transition (EMT) – probabilistic
        1. Only occurs if not in contact with Medium or any PERI cell compartment, but in contact with Mesench or Matrix
           1. Base EMT prob = 0.00001 (per cell per MCS)
           2. Increase EMT prob by Hill[TGFb3, 0.001, AC50=70]
           3. Decrease EMT prob by Hill[EGF, 0.001\*ahrDrivenEGFRFoldChange, AC50=70]
           4. Summary equation: 
           5. Set prob after a step of N MCS based on prob per MCS:

probN = 1 – (1 - prob)^N

* + - * 1. use random number generator to make decision
      1. If EMT decision made, change cell type from EPI\_\* to MESENCH\_EMT
    1. Decide whether an EPI cell differentiates into a PERI cell (EPT) – also probabilistic
       1. Only occurs if not in contact with Mesench or Matrix, but in contact with Medium or any PERI cell
          1. Base EPT prob = 0.0001 (per cell per MCS)
          2. If in direct contact with Medium (i.e. not covered by a PERI cell)

increase EPT prob by 0.01

* + - * 1. Set prob after a step of N MCS based on prob per MCS:

probN = 1 – (1 - prob)^N

* + - * 1. use random number generator to make decision
      1. If EPT decision made, change cell type from EPI\_\* to matching PERI\_\* type (e.g., EPI\_O -> PERI\_O)
  1. Growth
     1. All cell growth in the model stops at 3000 MCS
     2. Uses a growth prob and a growth rate for each cell
     3. For Mesench cells
        1. Base growth prob = 0.5
           1. Decreases to 0.1 if ephrinB1SignalFlag is false

i.e., Mesench cell not in contact with another Mesench cell

* + - 1. Base growth rate = 0 and max growth rate = 3\*0.4 = 1.2
         1. Increase growth rate based on concentration of free BMP2

Noggin binds to BMP2 and BMP4 and thus inhibits them from binding to their receptor

Fraction of free BMP: fBMP = ([BMP2] + [BMP4] – [Noggin])/([BMP2] + [BMP4])

Not allowed to go below zero

Form assumes strong binding (very low KD)

[Free BMP2] = [BMP2]\*fBMP

growth rate += Hill[free BMP2, 1.2, AC50=20]

* + - 1. Only allow growth if cell’s target volume < 1.5\*mitosis volume (no need to grow if already well above mitosis volume)
      2. Increase target volume by (growth rate)\*binomial(N, growth prob)
         1. Binomial function in python returns a random integer from 0 to N based on single success prob
         2. So form above picks a random number of steps in the last N=10 for which the cell grew and multiplies that number by growth rate to get change in target volume
      3. Set target surface = 4\*Sqrt(new target volume)
    1. For EPI cells
       1. Growth prob = 0.5
       2. Base growth rate = 0.016
          1. Increases by Hill[FGF10, 0.2, AC50=10]
          2. Increases by Hill[FGF7, 0.2, AC50=150]
          3. Decreases by Hill[TGFb3, 0.08, AC50=70, n=4]
          4. Increases by Hill[EGF, 0.08\* ahrDrivenEGFRFoldChange, AC50=70, n=4]
          5. Hill exponents > 1 show up in this EGF/TGFb3 effect, but not in apoptosis prob
       3. Only allow growth if cell’s target volume < 2.5\*mitosis volume (no need to grow if already well above mitosis volume)
       4. Increase target volume by (growth rate)\*binomial(N, growth prob)
          1. Binomial function in python returns a random integer from 0 to N based on single success prob
          2. So form above picks a random number of steps in the last N=10 for which the cell grew and multiplies that number by growth rate to get change in target volume
       5. Set target surface = 4\*Sqrt(new target volume)
    2. For basal PERI cells
       1. Growth prob = 0.5
       2. Base growth rate = 0.01
          1. Increases by Hill[FGF10, 0.12, AC50=10]
          2. Increases by Hill[FGF7, 0.12, AC50=150]
          3. Decreases by Hill[TGFb3, 0.08, AC50=70, n=4]
          4. Increases by Hill[EGF, 0.08\* ahrDrivenEGFRFoldChange, AC50=70, n=4]
          5. Hill exponents > 1 show up in this EGF/TGFb3 effect, but not in apoptosis prob
       3. Only allow growth if cell’s target volume < 2.5\*mitosis volume (no need to grow if already well above mitosis volume)
       4. Increase target volume of basal compartment by (growth rate)\*binomial(N, growth prob)
          1. Binomial function in python returns a random integer from 0 to N based on single success prob
          2. So form above picks a random number of steps in the last N=10 for which the cell grew and multiplies that number by growth rate to get change in target volume
       5. If basal PERI cell has a matching apical compartment…
          1. Set target surface of basal cell = 0.9\* (new target volume)
          2. Set target length of basal cell = 0.5\* (new target volume)
          3. Set target volume, surface area and length of apical compartment to match that of basal PERI cell
       6. If basal PERI cell does not have a matching apical compartment…
          1. Set target surface of basal cell = 0.7\* (new target volume)
          2. Set target length of basal cell = 0.3\* (new target volume)
  1. Matrix secretion/ingestion
     1. All matrix secretion/ingestion in the model stops at 3000 MCS
     2. Uses a matrix secretion prob and a matrix secretion rate from each cell
        1. Negative secretion rate = ingestion
     3. Matrix is only secreted by Mesench cells
        1. Base matrix secrete prob = 0.5
           1. Decreases to 0.25 if ephrinB1SignalFlag is false

i.e., Mesench cell not in contact with another Mesench cell

* + - 1. Matrix secretion rate governed by free BMP2
         1. Noggin binds to BMP2 and BMP4 and thus inhibits them from binding to their receptor
         2. Fraction of free BMP: fBMP = ([BMP2] + [BMP4] – [Noggin])/([BMP2] + [BMP4])

Not allowed to go below zero

Form assumes strong binding (very low KD)

* + - * 1. [Free BMP2] = [BMP2]\*fBMP
        2. matrix secretion rate = Hill[BMP2, 1.2, AC50=20]
      1. Determine volume to transfer from Mesench cell to Matrix “cell”
         1. vol to transfer = 1.2\*(-0.1N + 0.4\*binomial(N, secrete prob)

first term represents a constant rate of matrix ingestion or degradation

second term is a random # of steps in last N in which matrix is secreted

* + - * 1. limit vol to transfer to be < Mesench cell volume
      1. If there are adjacent Matrix “cells”
         1. Pick one at random
         2. Increase Matrix “cell’s” target volume by vol to transfer
         3. Decrease Mesench cell’s target volume by vol to transfer
         4. Set Mesench cell’s target surface = 4\*Sqrt(new target volume)
      2. If there is no adjacent Matrix “cell”
         1. Nucleate a new Matrix cell on surface of Mesench cell
         2. Set target volume of new Matrix cell = 1

Matrix lambda volume = 5\*0.5 = 2.5

* + - * 1. Decrease target volume of Mesench cell by 1
        2. Set Mesench cell’s target surface = 4\*Sqrt(new target volume)
  1. Basement membrane (BM) maintenance
     1. If an EPI cell is in direct contact with Mesench or Matrix
        1. Nucleate a pair of BM “cells” from adjacent points on border of this Epi cell with Mesench or Matrix (if Epi cell volume > 4)
        2. Nucleated BM pair has
           1. Same cluster ID
           2. Target volume = 3.5
           3. Lambda volume = 40\*(value for EPI cells) = 20
           4. No target surface or target length used
           5. Result is stiff “cells” that fluctuate only between 3-4 pixels
           6. Elastic FPP link rapidly forms between the nucleated pair
           7. Each member of pair can also form one FPP link with a BM “cell” with a different cluster ID

Result of internal and external FPP links is a chain of BM “cells”

* + 1. Model MMP action via probabilistic removal of BM cells
       1. Removal prob = 0.005\*[MMP]
       2. Use random number generator to decide whether to cleave/remove by setting target volume = 0
    2. If one member of a BM pair has been lost (via fluctuation or cleavage)
       1. Eliminate unmatched BM “cell” by setting target volume = 0
  1. Signal secretion
     1. By EPI and PERI cells (of all types unless otherwise noted)
        1. Initial secretion rates for first 100 MCS
           1. SHH secreted by medial EPI & PERI cells: rate = 10
           2. NOGGIN secreted by oral EPI & PERI cells: rate =10
           3. TGFb3 secreted by medial EPI & PERI cells: rate = 10
           4. Option for EGF secreted by medial EPI & PERI cells, but initial rate set to 0
        2. Condition dependent secretion rates in effect after 100 MCS
           1. SHH secreted by any EPI or PERI cell at rate dependent on [FGF10], [free BMP4] and [FGF7]

Basal secretion rate = 0

Increases by Hill[FGF10, 100, AC50=20]

Increases based on concentration of free BMP4

Noggin binds to BMP2 and BMP4 and thus inhibits them from binding to their receptor

Fraction of free BMP: fBMP = ([BMP2] + [BMP4] – [Noggin])/([BMP2] + [BMP4])

Not allowed to go below zero

Form assumes strong binding (very low KD)

[Free BMP4] = [BMP4]\*fBMP

secretion rate += Hill[free BMP4, 50, AC50=20]

Decreases by Hill[FGF7, 100, AC50=10]

Don’t allow rate to go below zero

* + - * 1. TGFb3 secreted by medial edge EPI & PERI cells at rate determined by [EGF]

Calculate concentration of bound EGF::EGFR

Basal [EGFR] = 130 (for one version)

Note that [EGFR] = (basal [EGFR])\* ahrDrivenEGFRFoldChange

[EGF::EGFR] = [EGF][EGFR]/(Kd + [EGF])

with Kd = 50

not the same as AC50 for EGF

secrete rate of TGFb3 = 15 + random[0,1] – Hill[EGF::EGFR, 15, AC50=70, n=4]

Part of EGF-TGFb3 mutual inhibition switch

* + - * 1. EGF secreted by medial edge EPI & PERI cells at rate determined by [TGFb3]

Basal [TGFbR] = 150 (for one version)

Calculate concentration of bound TGFb3::TGFbR

[TGFb3::TGFbR] = [TGFb3][TGFbR]/(Kd + [TGFb3])

with Kd = 50

not the same as AC50 for TGFb3

secrete rate of EGF = 15 + random[0,1] – Hill[TGFb3::TGFbR, 15, AC50=70, n=4]

Part of EGF-TGFb3 mutual inhibition switch

* + - * 1. MMP secreted by any EPI cell

Basal secretion rate = 1.0

Increases by Hill[TGFb3, 3.0, AC50=70, n=1]

* + - * 1. NOGGIN secreted by oral EPI & PERI cells

At constant rate = 10

* + 1. By Mesench cells (of all types unless otherwise noted)
       1. Initial secretion rates for first 100 MCS
          1. BMP4 secreted by all Mesench: rate = 30
          2. FGF7 secreted by nasal Mesench only: rate = 20
       2. Condition dependent secretion rates in effect after 100 MCS
          1. FGF10 and BMP2 secreted at rates dependent on [SHH] and ephrinB1 juxtacrine signaling (via contact with other Mesench cells)

Set max secrete rate

Default = 30

If no contact with other Mesench cells (ephrinB1Flag is false), reduce to 4

FGF10 secrete rate = Hill[SHH, maxSecreteRate, AC50=20]

BMP2 secrete rate = Hill[SHH, maxSecreteRate, AC50=20]

* + - * 1. BMP4 secreted at rate dependent on [free BMP4], [SHH] and ephrinB1 juxtacrine signaling

Set max secrete rate

Default = 30

If no contact with other Mesench cells (ephrinB1Flag is false), reduce to 4

Increases based on concentration of free BMP4

Noggin binds to BMP2 and BMP4 and thus inhibits them from binding to their receptor

Fraction of free BMP: fBMP = ([BMP2] + [BMP4] – [Noggin])/([BMP2] + [BMP4])

Not allowed to go below zero

Form assumes strong binding (very low KD)

[Free BMP4] = [BMP4]\*fBMP

secretion rate += Hill[free BMP4, 1.5\*maxSecreteRate, AC50=10]

Decreases by Hill[SHH, maxSecreteRate, AC50 = 20]

Not allowed to go below zero

* + - * 1. MMP secreted at a constant basal rate = 1.0
        2. FGF7 secreted by nasal Mesench only at rate dependent on [FGF7] and [SHH]

Self-activation increases rate by Hill[FGF7, 20, AC50=10]

Decreases by Hill[SHH, 20, AC50=20]

Not allowed to go below zero

* 1. Matrix elasticity
     1. Not used in current simulations
  2. Polarization of PERI cells
     1. Suppressed until MCS > 50
     2. If basal PERI cell has no matching apical compartment, nucleate one
        1. Pick a location along basal PERI cell’s border with Medium
        2. Set target volume of new apical compartment = 1.2\*(length of interface between basal compartment and Medium)
        3. Decrease target volume of basal compartment by same amount
        4. Set apical PERI target surface = its target volume
        5. Set basal PERI target surface = (0.7)\*(its new target volume)
        6. Set apical PERI target length = (0.5)\*its target volume
        7. Set basal PERI target length = (0.3)\*(its new target volume)
        8. Set lambda volume, surface and length of apical PERI to match basal
     3. If apical PERI cell has no matching basal compartment, nucleate one
        1. Pick a location along apical PERI cell’s border NOT in contact with Medium
        2. Set target volume of new basal compartment = 1.2\*( length of basal PERI border NOT in contact with Medium)
        3. Decrease target volume of apical compartment by same amount
        4. Set apical PERI target surface = its new target volume
        5. Set basal PERI target surface = (0.7)\*(its target volume)
        6. Set apical PERI target length = (0.5)\*(its new target volume)
        7. Set basal PERI target length = (0.3)\*(its target volume)
        8. Set lambda volume, surface and length of basal PERI to match apical
        9. An apical PERI cell without a matching basal PERI cell should be a very rare event until apoptosis starts in the medial edge seam (only a handful in each simulation)
     4. Code includes options for specialized tight junctions (TJ), but these are not currently used

What the text above means for apoptosis decisions of PERI\_\*a cells:



What the text above means for apoptosis decisions of PERI\_\* cells:



What the text above means for apoptosis decisions of EPI\_\* cells:



These are probabilities per cell per MCS.

OUTPUT FROM WT MODEL SHOWING AVG CELL VOLUMES AND MITOSIS VOLUMES

\*\*\*\*\* PalateModelCellBehaviors Steppable (start)

AVERAGE VOLUME AND SURFACE AREA OF EACH CELL TYPE

CellType AverageVolume AverageSurfaceArea

---------------------------------------------------------------

Wall 1196.0 1477.8

Mesench\_N 25.0 27.1

Mesench\_O 20.0 24.8

Epi\_N 28.0 31.2

Epi\_O 18.0 23.5

Epi\_ME 19.0 24.0

Peri\_N 43.0 43.4

Peri\_O 36.0 38.5

Peri\_ME 39.0 39.2

CellType MitosisVolume

------------------------------------

Medium 0.0

Wall 0.0

Mesench\_N 50.0

Mesench\_O 50.0

Mesench\_EMT 50.0

Matrix 0.0

Epi\_N 56.0

Epi\_O 56.0

Epi\_ME 84.0

BaseMemb 0.0

Peri\_N 86.0

Peri\_O 86.0

Peri\_ME 129.0

Peri\_Na 0.0

Peri\_Oa 0.0

Peri\_MEa 0.0

TJ 0.0

TJm 0.0

Assigning targetVolume and targetSurface of each cell to match that cell's initial volume and surface area.