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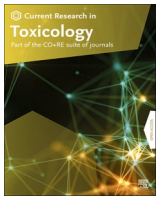

A computational dynamic systems model for *in silico* prediction of neural tube closure defects☆,☆☆

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ABSTRACT

Neural tube closure is a critical morphogenetic event during early vertebrate development. This complex process is susceptible to perturbation by genetic errors and chemical disruption, which can induce severe neural tube defects (NTDs) such as spina bifida. We built a computational agent-based model (ABM) of neural tube development based on the known biology of morphogenetic signals and cellular biomechanics underlying neural fold elevation, bending and fusion. The computer model functionalizes cell signals and responses to render a dynamic representation of neural tube closure. Perturbations in the control network can then be introduced synthetically or from biological data to yield quantitative simulation and probabilistic prediction of NTDs by incidence and degree of defect. Translational applications of the model include mechanistic understanding of how singular or combinatorial alterations in gene-environmental interactions and animal-free assessment of developmental toxicity for an important human birth defect (spina bifida) and potentially other neurological problems linked to development of the brain and spinal cord.

Introduction

Critical to embryonic development of the brain and spinal cord is elevation and folding of the neural plate into a neural tube. This process, neural tube closure, occurs between day 23 and day 30 of human gestation (Gilbert et al.). Its failure underlies neural tube defects (NTDs) such as spina bifida and anencephaly. With an estimated global incidence of 20 cases per 10,000 live-births, NTDs are among the most prevalent human congenital malformations (Kancherla, 2023). Individuals born with NTDs are known to have severe neurological and developmental problems.

Neural tube closure is a tightly regulated process that involves the coordinated action of various signaling pathways and cellular processes. This system is susceptible to disruptions, including genetic variations that alter the normal function of key genes involved in the process, and environmental factors such as chemical exposures (Kancherla, 2023; Heusinkveld et al., 2021; Nikolopoulou et al., 2017). Exposure to chemicals, such as pharmaceuticals, pesticides, and industrial chemicals, increases the risk of NTDs (Felisbino et al., 2024; Brender et al., 2014; Yazdy et al., 2013; Rull et al., 2006; Mulu et al., 2022; Lupo et al., 2011; Isaković et al., 2022; Huang et al., 2023). Therefore, these chemicals warrant attention in safety assessments traditionally conducted in guideline prenatal development studies, such as OECD 414 and OPPTS 870.3700 (OECD, 2018; EPA, 1998). However, there is an increasing demand for alternative approaches due to ethical concerns, the call for greater chemical coverage with fewer resources, and scientific arguments related to the limited predictability of animal models for human health (Kiani, et al., 2022; Piersma, 2006; Piersma et al., 2019).

Various *in vitro* alternatives for developmental toxicity testing have been developed during the past decades. These alternatives are often

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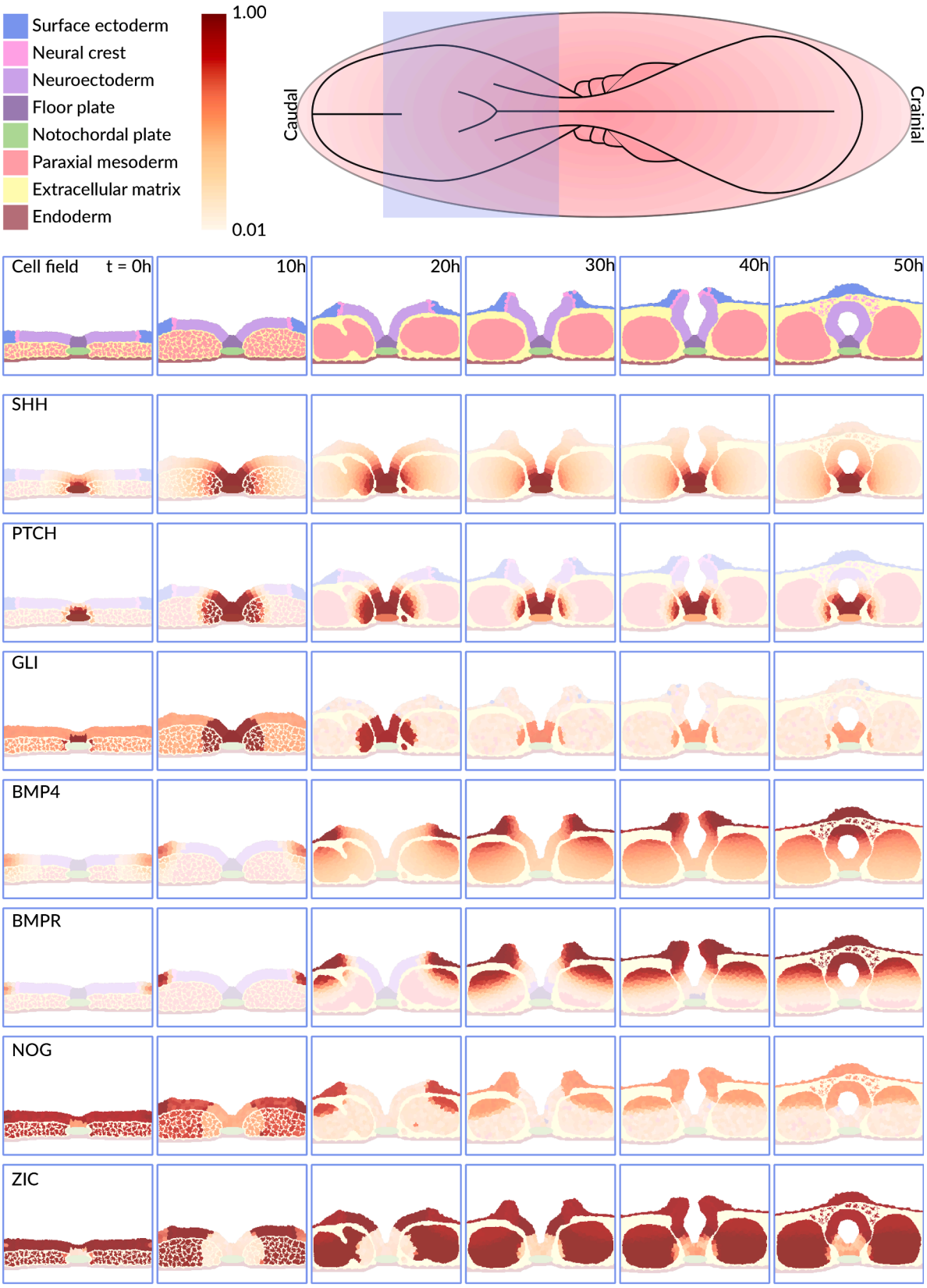

Fig. 1. A computational model of mammalian neural tube closure. The 8 different cell types featured in the model are incidated in legend with their respective color. The colorbar represents relative gene expression, where 1 indicates the highest amount and 0 the lowest amount. The model respresents a dorso-ventral section of the middle spinal region of the embryo, outlined by the violet box in the schematic. At t = 0 h the size of the model is 252 μm by 50 μm by 1. The gene that is visualized in each image series is indicated in the top left corner of the first image of the series. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

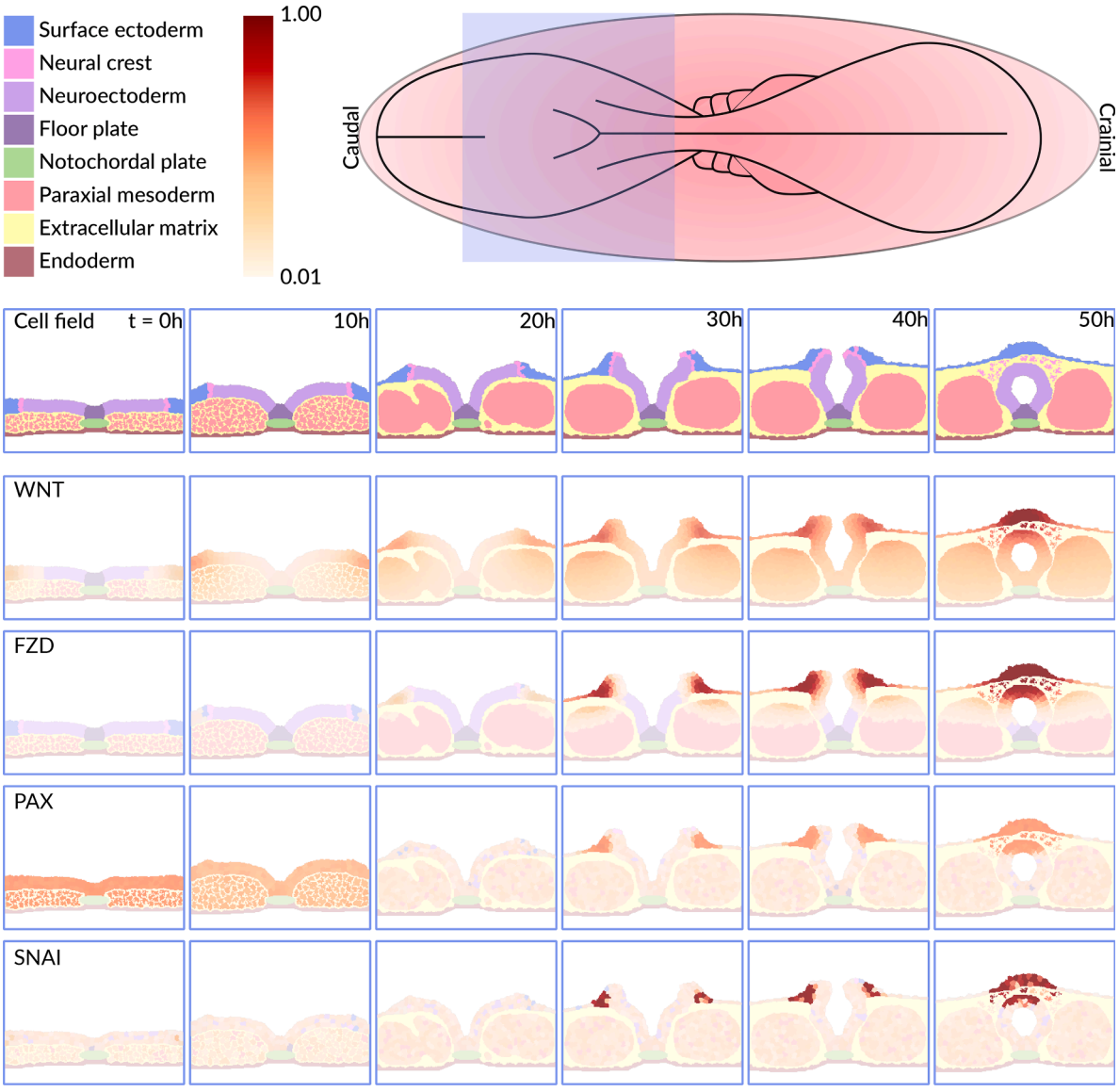

Fig. 1. (*continued*).

based on pluripotent stem cells, small model organisms such as the zebrafish, and complex organoid or *in vitro* engineered microsystems (Xue et al., 2018; Lee et al., 2010; Dreser et al., 2020; McCollum et al., 2011; Van Der Ven et al., 2022; Karzbrun et al., 2021; Xue et al., 2024; Knight et al., 2018). These approaches may replicate some key aspects of neural tube development (e.g., neuruloids, neural rosettes), offering a window into the potential teratogenic effects leading to NTDs. Despite advancements, it is still challenging to capture the complex interactions and integrative biology of *in vivo* mammalian development.

Computational agent-based models (ABMs) are a method in which individual entities, or agents—specifically cells in this context—are simulated to mimic and study complex behaviors. These models put cellular dynamics in motion to reproduce self-organizing and emergent phenotypes designed in a computer model to enable the prediction of systems-level outcomes linked to defined biomolecular abnormalities (Hutson et al., 2017; Kleinstreuer et al., 2013; Swat et al., 2012; Shirinifard et al., 2012). For example, the models developed for blood vessel formation and palatal fusion have demonstrated how genetic and chemical perturbations, represented by adjusted fold changes in key genes, can predict adverse outcomes observed *in vivo* (Hutson et al., 2017; Kleinstreuer et al., 2013). This approach provides valuable understanding of the mechanisms and vulnerabilities of these developmental processes. When designed with sufficient biological accuracy, ABMs can probabilistically assess the impact of perturbations on neural tube closure. This offers insights into mechanisms underlying neural tube closure and potential disruption of this process by chemical or genetic factors.

In this study, we present a new computational ABM that recapitulates the morphogenetic events driving mammalian neural tube closure, using the open source CompuCell3D modeling environment (Swat et al., 2012). Our ABM is based on the physiological map of mammalian neural tube closure we developed earlier, which systematically organizes the cellular and molecular interactions driving this process (Heusinkveld et al., 2021). This map serves as a reference tool for identifying critical pathways and integrating them into the ABM. Here, we show how variations in cell signals and responses may lead to alterations reminiscent of NTDs that can be calibrated to *in vivo* models (mouse, zebrafish). Additionally, the computer model can offer mechanistic insights often obscured in traditional animal models to bridge the gap between a molecular alteration and a developmental phenotype.

Results

A computational model of the mammalian neural tube closure

We built a multi-scale model of mammalian neural tube closure using CompuCell3D, an open-source platform that simulates multicellular systems based on a Cellular Potts model. The platform takes into account the specific behaviors of cells, their regulatory signals, and physical properties (Swat, et al., 2012). Neural tube closure involves complex tissue morphogenesis where tissue bending plays an essential role. This bending is primarily driven by apical constriction of cells in the neuroepithelium. The collective changes shape the tissue at emergent hinge points (bending points) (Nikolopoulou et al., 2017). The current model is two-dimensional in the dorso-ventral plane representing the mid-lumbar region of the neural tube. Within this region, both median hinge points (MHPs) and dorsolateral hinge points (DLHPs) are present during neural tube closure (Juriloff and Harris, 2018).

The stepwise progression of the model is shown in Fig. 1. It starts as a planar neural plate, measuring 252 μm by 50 μm by 1 μm, and over the course of 50 simulated hours undergoes the morphogenetic changes necessary to form a closed neural tube (Cork and Gasser, 2012). The start of the model approximates gestational day 28, Carnegie Stage 10 in human development. To make the model as human-like as possible, we adjusted its physical properties, time constraints and spatial organization to closely represent the characteristics of human neural tube closure (Cork and Gasser, 2012). Executing the computer model simulates key morphogenetic events akin to those seen in reconstructed sections of the embryo (Cork and Gasser, 2012). These events are directly driven by a gene regulatory network that is embedded in each cell featured in the ABM (Fig. 2). Below we describe the biological processes that occur during neural tube closure and are incorporated in our model, which progress through six distinct stages.

In the first stage, molecular protein signals shape the initial morphogenesis. The notochord starts to produce sonic hedgehog (SHH), which activates Patched Homolog 1 (PTCH1) (Aruga, 2004; Caspary and Anderson, 2003). PTCH1 downregulates Zinc finger proteins (ZIC) and upregulates Glioma-associated oncogene 2 (GLI2) proteins, which inhibit ZIC (Fig. 2) (Aruga, 2004; Caspary and Anderson, 2003; Copp and Greene, 2010). This starts apical constriction and the formation of median hinge-point cells in the targeted region (Caspary and Anderson, 2003; Copp and Greene, 2010). Opposing the SHH gradient is the Bone morphogenetic protein 4 (BMP4) gradient, secreted by the surface ectoderm (Copp and Greene, 2010; Liu and Niswander, 2005; Sheng et al., 2010; Anderson et al., 2016). BMP4 induces cell proliferation in the mesoderm and activates the BMP receptor (BMPR) (Sheng et al., 2010; Anderson et al., 2016; Chesnutt et al., 2004; Sakurai et al., 2012). BMP4 activity is regulated by fibroblast growth factor 8 (FGF8) and Noggin (NOG). FGF8, which levels are highest in the first stage, directly inhibits BMP4 gene expression (Fig. 2). NOG, secreted by the mesoderm, neuroectoderm, and notochord, acts as a ligand that binds to BMP4, forming an inactive NOG-BMP4 complex (Fig. 2) (Felder et al., 2002; Phan-Everson et al., 2021; Zimmerman et al., 1996). The surface ectoderm also secretes WNT, which activates the Frizzled (FZD) receptor to start the cascade of events leading to neural crest cell delamination (Nikolopoulou et al., 2017; Gray et al., 2013; Pera et al., 2014). However, early neural crest specification and delamination is inhibited by the high levels of FGF8 (Diez Del Corral and Morales, 2017). This is complemented by low levels of all-trans retinoic acid (ATRA), FGF8′s regulatory counterpart (Heusinkveld et al., 2021).

In the second stage, approximately 10 h into neural tube

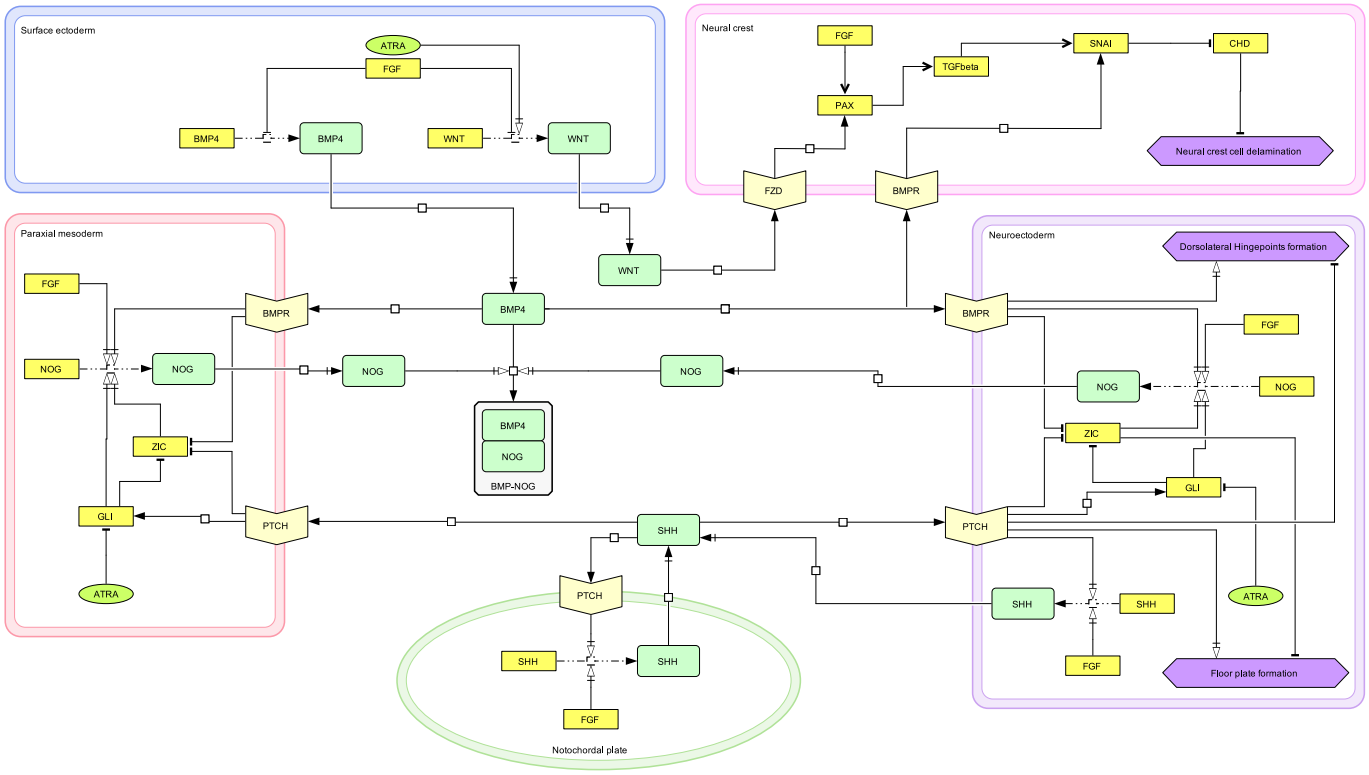

Fig. 2. Gene regulatory network that drives the neural tube closure model. The network regulates three key processes crucial for successful neural tube closure (purple diamonds): floor plate formation, dorsolateral hinge point formation, and neural crest cell delamination. Symbols represent specific molecular and cellular components within the network: light-yellow chevrons indicate receptors, yellow rectangles represent genes, and green rectangles depict proteins. Arrows and their formatting denote specific interactions: white arrows represent specific triggering events; black arrows indicate receptor binding; and black arrows with a line signify transport processes. White squares provide anchor points for the attachment of trigger arrows to reaction pathways, enhancing diagram clarity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

development, the median hinge point cells anchor to the notochord beneath them and start to form the floor plate. The floor plate gives stability to the neural plate and starts to produce SHH, reinforcing the sustained SHH gradient along the ventral-dorsal axis (Greene et al., 1998). Floor plate formation in combination with further growth of the mesoderm results in the formation of a furrow at the midline of the model, the neural groove (van Straaten et al., 1993). Meanwhile, physical forces outside of the neural plate are also at work. The surface ectoderm starts to push towards the midline of the neural tube (Schoenwolf and Alvarez, 1991). These forces are generated by the pressure of surrounding tissues or fluids and are critical for bending of the neural plate later in time. In our simulation, we approximated this behavior by applying a directional vector force on the surface ectoderm, which compels the surface ectoderm to move inward. This ensures that the neural tube invaginates into the embryo and not outward (Schoenwolf and Alvarez, 1991).

In the third stage, around 20 h into development, key molecular changes occur. ATRA levels continue to increase, exceeding FGF levels, while FGF levels further decrease (Heusinkveld et al., 2021). This shift results in reduced NOG levels, subsequently increasing the amount of free active BMP4. Still, the NOG-BMP4 balance is maintained because BMP4 induces the production of NOG (Felder et al., 2002; Phan-Everson et al., 2021). Somitogenesis commences and plays a critical role in shaping and stabilizing the neuroectodermal tissue. This contributes to the elevation of the neural folds by exerting physical forces that push up the neuroectoderm, thereby deepening the neural groove (Christodoulou and Skourides, 2023). During neural tube closure, somite formation is initiated via an oscillatory mechanism, often referred to as the "segmentation clock", in which the Notch, WNT and FGF pathways play a major role (Gibb et al., 2010; Maroto et al., 2012). The computer model abstracts a segmentation clock without its regulators, as noted in the Methods section.

Around 30 h into development, the hallmark of the fourth stage is marked by the formation of dorsolateral hinge-points (DLHPs) at the neural plate's upper regions. DLHP formation initiates bending of the neural plate via apical constriction, the formation of wedge-shaped cells. This process is driven by localized actomyosin contraction at the apical side of cells and is accompanied by cell elongation, as cell volume is redistributed along the apicobasal axis (Sawyer et al., 2010). Basal thickening, with nuclei retained basally, further reinforces this wedge shape and stabilizes the hinge point structure. DLHP formation is highest in regions with intermediate BMP4 levels, where BMP signaling is inhibited by NOG, ensuring proper spatial localization (Gilbert et al.). In contrast, SHH signaling via PTCH acts as an inhibitor of DLHP formation, further refining their placement (Stottmann et al., 2006; Ybot-Gonzalez et al., 2007). At this stage, the continuous downregulation of FGF and increasing activity of ATRA allows the start of the neural crest cell delamination cascade. The end of the cascade results in an E- to N-cadherin switch, which allows neural crest cells to delaminate. In our model we simplified this through a single Cadherin (CAD). When CAD expression is high, its properties approximate E-cadherin. Low expression switches to N-cadherin properties. The E/N Cadherin switch is inhibited by FGF8 and induced by BMP4, FZD, paired box gene 3 (PAX3) and snail family transcriptional repressor 1 (SNAI1), which is increasing in this stage (Fig. 1) (Gray et al., 2013; Pera et al., 2014; Ichi et al., 2010; Nakazaki et al., 2009).

In the fifth stage, around 40 h into development, the formation of DLHPs further progressed into the center region of the neural plate under the influence of an increasing BMP4 gradient (Sheng et al., 2010; Anderson et al., 2016). This change, combined with the physical forces exerted by the surface ectoderm towards the midline, leads to near fusion of the surface ectoderm and neuroectoderm, almost closing the neural tube (Schoenwolf and Alvarez, 1991). The rising FZD, PAX3 and SNAI1 levels cause the start of the neural crest cell delamination (Gray et al., 2013; Pera et al., 2014; Ichi et al., 2010; Nakazaki et al., 2009).

After approximately 40–50 h of development, a section of the neural tube closes. The final stage displays 50 h into development, the neural tube is now fully closed, and the neural crest cells delaminated. A summary of all the signaling molecules and cell behaviors implemented in the computational model is presented in Table 1.

Table 1
Summary of the molecules and behavior in the neural tube closure model.

Cell type	Behavior	Signal molecules	Reference
Surface Ectoderm	BMP4 secretion, WNT secretion, cell migration	BMP4, FGF8, ATRA, WNT	(Liu and Niswander, 2005; Sheng, 2010; Anderson et al., 2016)
Neural crest	cell delamination	WNT, FZD, FGF8, PAX3, TGFbeta, SNAI1, CHD, BMP4, BMPR	(Gray, 2013; Pera et al., 2014; Ichi, 2010; Nakazaki, 2009)
Neuroectoderm	apical constriction, DLHP formation, floor plate formation, NOG secretionSHH secretion	NOG, FGF8, BMP4, BMPR, ZIC, GLI2, ATRA, SHH, PTCH	(Greene et al., 1998; van Straaten et al., 1993; Caspary and Anderson, 2003; Copp and Greene, 2010; Felder, 2002; Phan-Everson, 2021; Zimmerman et al., 1996)
Mesoderm	cell proliferation, somite formation, NOG secretion	NOG, FGF8, BMP4, BMPR, ZIC, GLI2, ATRA, SHH, PTCH	(Anderson et al., 2016; Chesnutt et al., 2004; Sakurai, 2012; Felder, 2002; Phan-Everson, 2021; Zimmerman et al., 1996)
Notochordal plate	SHH secretion, Floor plate induction	SHH, PTCH, FGF8	(Aruga, 2004; Caspary and Anderson, 2003)

Towards modeling neural tube closure defects

To calibrate our computational model against known biological data and explore potential developmental anomalies, we performed *in silico* parameter variation experiments. In these experiments nine different genes that participate in the regulation of the three key processes (floor plate formation, DLHPs formation, neural crest cell delamination) were hyperactivated or knocked out, while keeping the rest of the parameters the same. Each hyperactivation or knockout scenario was simulated 20 times to calculate the probability of defect, with the standard error of the mean (SEM) calculated to represent variability across simulations. The results presented in the following sections are organized per morphogenetic event and offer insight in the predicted dynamics of neural tube closure. The series of images shown are examples of the most common outcomes (Fig. 3). A summary of the *in silico* gene perturbation experiments is presented in Supplementary Table 1.

Floor plate formation

The floor plate is a specialized structure of the neural tube and is formed in the second stage of the neural tube closure. Floor plate formation was disrupted in the model by knockout of *SHH*, *PTCH1* or *GLI2* (Fig. 3). This prevented the neuroectoderm from anchoring to notochord, which reduced the stability of the neural plate. As a result, structural malformations occurred at the midline of the neural tube.

Due to disruptions in floor plate formation, the strength of the SHH gradient along the ventral-dorsal axis was reduced. Due to the reduced SHH gradient (the inhibitor of DLHP formation), DLHP formation was increased near the ventral side of the tube. Due to increased DLHPs formation, the neural tube still managed to close in most cases. However, the lack of floor plate and reduced stability of the neuroectoderm reduced the size of the neural canal (the hollow part of the neural tube).

energy function. Specifically, changes that improve the pseudo energy always succeed, while those that worsen it may succeed based on a probability linked to the effective "temperature" (Teff), which was set to 10 in our simulations. This value was chosen based on recommendations in the CompuCell3D manual to balance stochasticity and energy minimization. This temperature introduces randomness in cellular motility and behavior, ensuring the model captures biological variability while preserving realistic dynamics (Swat et al., 2012). Exact details of the modeling framework can be found in the Compucell3D manual on compucell3d.org and in Shirinfiard et al. 2012 (Shirinifard et al., 2012). We quantified the dynamics in terms of Monte Carlo Steps (MCS), with each MCS involving 252 * 200 = 50400 change attempts. Each simulation ran for a total of 12.000 Monte Carlo Steps (MCS), with one MCS representing 15 s real time for a total real time of 50 h. Up to 20 simulations were simultaneously ran locally on a 3.60 GHz intel core i7-12700KF processor, which took approximately 2 h to complete. We stored cell and field-lattice configurations every 25 simulated minutes (100 MCS) and rendered the snapshots using a home-written python script (Figs. 1 and 3).

Model scope

Our model recapitulates a single dorso-ventral section of caudal neural tube closure, specifically the middle spinal region at somite locations 15–20. The model covers major morphogenetic events that revolve around the all-*trans*-retinoic acid (ATRA)-related molecular pathways for neural tube closure and disruption (Heusinkveld et al., 2021). These events include apical constriction, Floor plate formation, DLHPs formation, neural fold elevation, neural crest cell delamination, surface ectoderm fusion and somite formation. Here Floor plate formation, DLHPs formation and neural crest cell delamination are actively regulated by a constructed gene regulatory network (Fig. 2). The model approximates a timeline from embryonic (E) days E8.5 − E10 in the mouse and gestational days 28–31, Carnegie Stage (CS) CS10 − CS12 in human development (Cork and Gasser, 2012). Initial model size of 252 x 50 was set based on CS10 sections of the caudal neural tube at the virtual human embryo project (Cork and Gasser, 2012).

Model cell properties

We initialized the cell volume for the paraxial mesoderm, surface ectoderm, and neural crest cell types at 49, with a variance of plus or minus 10, and positioned them in a 7 by 7 μm layout. This setup mirrors observations from the Virtual Human Embryo project (Cork and Gasser, 2012). We calibrated the delta cell volume increase to reflect a 24-hour proliferation rate without inducing cell proliferation. For the neuroectoderm and median hinge point cell types, we divided each cell into basal, lateral, and apical sites, each spanning 7 by 7 μm. These cells, as observed in the Virtual Human Embryo images, seemed elongated, being roughly three times longer than other cell types. To keep the neural plate structure stable and intact during the simulation, a minimum cell width of 7 μm was essential, making our simulated neuroectoderm cells approximately 2 μm wider than their estimated biological size. We ensured the structural integrity of the neural plate by connecting apical and basal cells within the neuroectodermal layer with springs, simulating the role of tight junctions through the use of the Focal Point Plasticity plugin in CompuCell3D (Swat et al., 2012). We set the target distance between these points at 5 μm to create a stable neuroectodermal layer.

Model cell behavior

Apical Constriction in MHP formation: Apical constriction in the MHP is directly induced by SHH signaling secreted by the notochordal plate. When neuroectodermal cells are in contact with the notochord, the high SHH signaling triggers apical constriction via PTCH (Fig. 2). When a cell undergoes apical constriction, the target distance of the mechanical springs on the apical side of the cell reduces from 5 μm to 0 μm, implemented in steps of 0.005 μm per MCS for stable dynamic transition. This reduction represents the constriction of the actomyosin machinery on the apical surface of the cell (Cork and Gasser, 2012; Greene et al., 1998; Sawyer et al., 2010). To simulate the elongation of cells during apical constriction, additional mechanical springs are introduced between the apical and basal sides of the cell. These springs extend the target distance of the basal compartment to 15 μm from an initial 9 μm, reflecting the elongation behavior during apical constriction (Cork and Gasser, 2012; Sawyer et al., 2010). Simultaneously, cell volume is dynamically redistributed among three compartments (apical, lateral, and basal), increasing the basal compartment's volume by 57 % while proportionally decreasing the apical compartment's volume. This adjustment mimics basal thickening and the formation of wedge-shaped cells while maintaining the overall cell volume (Gilbert et al.; Cork and Gasser, 2012).

Apical Constriction during DLPHs formation: Apical constriction during DLHPs formation in the neuroectoderm is an emergent feature induced by intermediate BMP4 levels and inhibited by SHH (Fig. 2). While the behavior of cells undergoing apical constriction during DLHP formation resembles that during MHP formation, the triggering mechanisms differ. the first model calculates the maximum probability of apical constriction (max_{ac}) for each neuroectodermal cell at every MCS based on local BMP4 concentration using the equation:

$$max_{ac} = 4\left(\frac{C_{bmp}^8}{0.5^8 + xC_{bmp}^8} * \frac{0.5^8}{0.5^8 + C_{bmp}^8}\right) \quad (1)$$

Here max_{ac} is the maximum apical constriction probability and C_{bmp} is the local BMP4 concentration. Next the inhibition of apical constriction by SHH (shh_{aci}) is calculated using the equation:

$$shh_{aci} = \frac{0.5^8}{0.5^8 + C_{shh}^8} \quad (2)$$

where shh_{aci} represents represents the level of apical constriction inhibition by SHH and C_{shh} is the local SHH concentration. The combined probability of apical constriction ($rate_{ac}$) is then determined by multiplying the BMP4 and SHH effects:

$$rate_{ac} = max_{ac} * \mathrm{shh}_{\mathrm{aci}} \quad (3)$$

This value ($rate_{ac}$) is subsequently corrected for time using a Poisson distribution:

$$p_{ac} = 1 - \exp^{-\left(\frac{rate_{ac}}{4}\right)} \quad (4)$$

Here p_{ac} represents the time-corrected probability of apical constriction. Upon successful apical constriction, the cell transitions one step closer to adopting a wedge-shaped morphology. If unsuccessful, the cell reverts one step towards its original shape. This stochastic approach captures the biological variability and dynamic nature of DLHP formation.

The segmentation clock: In our model, we implemented the segmentation clock via an internal "counter," that triggers the start of somite formation after 17 h simulated time (Cork and Gasser, 2012). Cells from the paraxial mesoderm type have a 1/2500 chance to differentiate to a somite cell with different properties. This probability is increased by 1 %, each time a paraxial mesoderm cell contacts a somite cell. These parameters were arbitrarily chosen to ensure rapid somite formation while minimizing the amount of extracellular matrix (ECM) captured within these somites (Martins et al., 2009). This implementation allowed us to reduce the complexity of the model while maintaining the critical function of the somites in neural tube closure.

A gene regulatory network that drives the neural tube closure model

To simulate the complex interplay between various genes and proteins during neural tube closure in our model, we created a gene regulatory network of neural tube closure that contains key genes. All genes and proteins implemented in the model are displayed in the gene regulatory network (Fig. 2). The gene regulatory network implemented in our model is a refined extraction from a larger network and embedded in each cell in the ABM (Heusinkveld et al., 2021). Our network regulates three key processes for successful neural tube closure: (Fig. 2) floor plate formation, DLHP formation and neural crest cell delamination (Fig. 2). The development and simulation of this gene regulatory network, was conducted entirely within the CompuCell3D environment. As mechanistic data of the human neural tube is scarce the network primarily relies on animal data.

$$gene_a = \frac{x^n}{K^n + x^n} \tag{5}$$

$$gene_i = 1 - \frac{K^n}{K^n + x^n} \tag{6}$$

In these equations:

- x represents the gene expression level of the regulating gene (activator or inhibitor).
- K is the half-maximal constant.
- n is the Hill coefficient.

The Hill functions in the network are scaled between 0 and 1, with $n = 8$ and $K = 0.5$. These parameter values were selected due to the absence of precise values in existing data. To incorporate the inherent variability and unpredictability of biological systems, a random background transcription level is included for each gene in the network.

Protein gradients that are known to be critical for neural tube include BMP secreted by the surface ectoderm, SHH from the notochord and floor plate, NOG from the mesoderm, neuroectoderm, and floor plate, and WNT from the surface ectoderm. The protein concentration of these gradients is scaled between 0 and 1, a similar scaling as the gene regulatory network. We normalized the protein gradients by selecting identical values for each gradient for the diffusion and decay constant. The diffusion constant for all protein gradients was parameterized to be 0.24 μm (Kancherla, 2023)/s. The decay constant was parameterized to be $3.33*10^{-5}s^{-1}$ in all compartments but increased to $2.333*10^{-4}s^{-1}$ in the medium compartment (all values scaled to the simulation's time and space units). This ensured consistent behavior of these signaling molecules across different cell types and spatial locations. Then, we calibrated the secretion constants based on cell number to accurately reflect the biological context (exact parameter values can be found in the simulation's parameter file). The rostro-caudal gradients: ATRA and FGF are fixed in space, meaning their concentrations are the same throughout the model, but vary over time. Specifically, FGF concentration decreases over time and ATRA increases, both scaled between 0 and 1. This design mimics the rostro-caudal protein balance of FGF-ATRA crucial for neural tube closure, and ensures that these gradients capture the temporal dynamics needed for patterning in this 2D framework (Heusinkveld et al., 2021).

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CRediT authorship contribution statement

Job H. Berkhout: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **James A. Glazier:** Conceptualization, Methodology, Software, Validation, Supervision. **Aldert H. Piersma:** Conceptualization, Project administration, Funding acquisition, Writing – review & editing. **Julio M. Belmonte:** Methodology, Software. **Juliette Legler:** Project administration, Funding acquisition, Writing – review & editing. **Richard M. Spencer:** Conceptualization, Methodology, Software. **Thomas B. Knudsen:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing. **Harm J. Heusinkveld:** Conceptualization, Validation, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crtox.2024.100210.

Data availability

All data generated during this study is available in the BioStudies database (http://www.ebi.ac.uk/biostudies) under accession number S-ONTX34.

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    <Energy Type1="ECM" Type2="notochord">7.0</Energy>
    <Energy Type1="ECM" Type2="somite">7.0</Energy>
    <Energy Type1="ECM" Type2="mesoderm">0.0</Energy>
    <Energy Type1="ECM" Type2="mhp_basal">10.0</Energy>
    <Energy Type1="ECM" Type2="mhp_lateral">20.0</Energy>
    <Energy Type1="ECM" Type2="mhp_apical">25.0</Energy>
    <Energy Type1="ECM" Type2="np_basal_L">5.0</Energy>
    <Energy Type1="ECM" Type2="np_lateral_L">10.0</Energy>
    <Energy Type1="ECM" Type2="np_apical_L">20.0</Energy>
    <Energy Type1="ECM" Type2="np_basal_R">5.0</Energy>
    <Energy Type1="ECM" Type2="np_lateral_R">10.0</Energy>
    <Energy Type1="ECM" Type2="np_apical_R">20.0</Energy>
    <Energy Type1="ECM" Type2="ectoderm_L">10.0</Energy>
    <Energy Type1="ECM" Type2="ectoderm_R">10.0</Energy>
    <Energy Type1="ECM" Type2="Medium">50.0</Energy>
    <Energy Type1="ECM" Type2="neuralcrest_L">10.0</Energy>
    <Energy Type1="ECM" Type2="neuralcrest_R">10.0</Energy>
    <Energy Type1="ECM" Type2="neuralcrest_L_DEL">0.0</Energy>
    <Energy Type1="ECM" Type2="neuralcrest_R_DEL">0.0</Energy>

    <Energy Type1="fluid" Type2="fluid">1.0</Energy>
    <Energy Type1="fluid" Type2="endoderm">10.0</Energy>
    <Energy Type1="fluid" Type2="notochord">10.0</Energy>
    <Energy Type1="fluid" Type2="somite">30.0</Energy>
    <Energy Type1="fluid" Type2="mesoderm">10.0</Energy>
    <Energy Type1="fluid" Type2="mhp_basal">10.0</Energy>
    <Energy Type1="fluid" Type2="mhp_lateral">10.0</Energy>
    <Energy Type1="fluid" Type2="mhp_apical">10.0</Energy>
    <Energy Type1="fluid" Type2="np_basal_L">10.0</Energy>
    <Energy Type1="fluid" Type2="np_lateral_L">10.0</Energy>
    <Energy Type1="fluid" Type2="np_apical_L">10.0</Energy>
```

```
      <Energy Type1="fluid" Type2="np_basal_R">10.0</Energy>
      <Energy Type1="fluid" Type2="np_lateral_R">10.0</Energy>
      <Energy Type1="fluid" Type2="np_apical_R">10.0</Energy>
      <Energy Type1="fluid" Type2="ectoderm_L">10.0</Energy>
      <Energy Type1="fluid" Type2="ectoderm_R">10.0</Energy>
      <Energy Type1="fluid" Type2="ECM">10.0</Energy>
      <Energy Type1="fluid" Type2="Medium">10.0</Energy>
      <Energy Type1="fluid" Type2="wall">0.0</Energy>


      <NeighborOrder>4</NeighborOrder>
   </Plugin>



   <Plugin Name="ContactInternal">
      <!-- Specification of internal adhesion energies -->

      <Energy Type1="mhp_basal" Type2="mhp_lateral">4.0</Energy>
      <Energy Type1="mhp_basal" Type2="mhp_apical">4.0</Energy>
      <Energy Type1="mhp_basal" Type2="np_lateral_L">4.0</Energy>
      <Energy Type1="mhp_basal" Type2="np_apical_L">4.0</Energy>
      <Energy Type1="mhp_basal" Type2="np_lateral_R">4.0</Energy>
      <Energy Type1="mhp_basal" Type2="np_apical_R">4.0</Energy>

      <Energy Type1="mhp_lateral" Type2="mhp_apical">4.0</Energy>
      <Energy Type1="mhp_lateral" Type2="np_basal_L">4.0</Energy>
      <Energy Type1="mhp_lateral" Type2="np_apical_L">4.0</Energy>
      <Energy Type1="mhp_lateral" Type2="np_basal_R">4.0</Energy>
      <Energy Type1="mhp_lateral" Type2="np_apical_R">4.0</Energy>

      <Energy Type1="mhp_apical" Type2="np_basal_L">4.0</Energy>
      <Energy Type1="mhp_apical" Type2="np_lateral_L">4.0</Energy>
      <Energy Type1="mhp_apical" Type2="np_basal_R">4.0</Energy>
      <Energy Type1="mhp_apical" Type2="np_lateral_R">4.0</Energy>

      <Energy Type1="np_basal_L" Type2="np_lateral_L">4.0</Energy>
      <Energy Type1="np_basal_L" Type2="np_apical_L">4.0</Energy>
      <Energy Type1="np_basal_L" Type2="np_lateral_R">4.0</Energy>
      <Energy Type1="np_basal_L" Type2="np_apical_R">4.0</Energy>

      <Energy Type1="np_lateral_L" Type2="np_apical_L">4.0</Energy>
      <Energy Type1="np_lateral_L" Type2="np_basal_R">4.0</Energy>
      <Energy Type1="np_lateral_L" Type2="np_lateral_R">0.0</Energy>
      <Energy Type1="np_lateral_L" Type2="np_apical_R">4.0</Energy>

      <Energy Type1="np_apical_L" Type2="np_basal_R">4.0</Energy>
      <Energy Type1="np_apical_L" Type2="np_lateral_R">4.0</Energy>

      <Energy Type1="np_basal_R" Type2="np_lateral_R">4.0</Energy>
      <Energy Type1="np_basal_R" Type2="np_apical_R">4.0</Energy>

      <Energy Type1="np_lateral_R" Type2="np_apical_R">4.0</Energy>

      <NeighborOrder>4</NeighborOrder>
   </Plugin>

   <Plugin Name="FocalPointPlasticity">

      <!-- Specification of focal point junctions -->
      <!-- We separetely specify links between members of same cluster - InternalParameters and
```

```
members of different clusters Parameters. When not using compartmental  cells comment out
InternalParameters specification -->
<!-- To modify FPP links individually for each cell pair uncomment line below -->
<Local/>
<!-- Note that even though you may manipulate lambdaDistance, targetDistance and
maxDistance using Python you still need to set activation energy from XML level -->
<!-- See CC3D manual for details on FPP plugin  -->

<Parameters Type1="mhp_apical" Type2="mhp_apical">
   <Lambda>20</Lambda>
   <ActivationEnergy>-50</ActivationEnergy>
   <TargetDistance>5</TargetDistance>
   <MaxDistance>20</MaxDistance>
   <MaxNumberOfJunctions NeighborOrder="1">2</MaxNumberOfJunctions>
</Parameters>


<Parameters Type1="mhp_apical" Type2="np_apical_R">
   <Lambda>20</Lambda>
   <ActivationEnergy>-50</ActivationEnergy>
   <TargetDistance>6</TargetDistance>
   <MaxDistance>10</MaxDistance>
   <MaxNumberOfJunctions NeighborOrder="1">1</MaxNumberOfJunctions>
</Parameters>


<!-- <Parameters Type1="np_apical_R" Type2="np_apical_R"> -->
   <!-- <Lambda>20</Lambda> -->
   <!-- <ActivationEnergy>-50</ActivationEnergy> -->
   <!-- <TargetDistance>5</TargetDistance> -->
   <!-- <MaxDistance>20</MaxDistance> -->
   <!-- <MaxNumberOfJunctions NeighborOrder="1">2</MaxNumberOfJunctions> -->
<!-- </Parameters> -->

<Parameters Type1="mhp_basal" Type2="mhp_basal">
   <Lambda>20</Lambda>
   <ActivationEnergy>-50</ActivationEnergy>
   <TargetDistance>6</TargetDistance>
   <MaxDistance>20</MaxDistance>
   <MaxNumberOfJunctions NeighborOrder="1">2</MaxNumberOfJunctions>
</Parameters>

<Parameters Type1="mhp_basal" Type2="np_basal_R">
   <Lambda>20</Lambda>
   <ActivationEnergy>-50</ActivationEnergy>
   <TargetDistance>5</TargetDistance>
   <MaxDistance>20</MaxDistance>
   <MaxNumberOfJunctions NeighborOrder="1">1</MaxNumberOfJunctions>
</Parameters>


<Parameters Type1="np_basal_R" Type2="np_basal_R">
   <Lambda>20</Lambda>
   <ActivationEnergy>-50</ActivationEnergy>
   <TargetDistance>5</TargetDistance>
   <MaxDistance>20</MaxDistance>
   <MaxNumberOfJunctions NeighborOrder="1">2</MaxNumberOfJunctions>
</Parameters>

<Parameters Type1="endoderm" Type2="endoderm">
   <Lambda>20</Lambda>
```

```
      <ActivationEnergy>-75</ActivationEnergy>
      <TargetDistance>14</TargetDistance>
      <MaxDistance>30</MaxDistance>
      <MaxNumberOfJunctions NeighborOrder="1">2</MaxNumberOfJunctions>
   </Parameters>


   <NeighborOrder>1</NeighborOrder>
</Plugin>

<Plugin Name="Secretion">

   <!-- Specification of secretion properties of select cell types. -->
   <!-- You may repeat Field element for each chemical field declared in the PDE solvers -->
   <!-- Specification of secretion properties of individual cells can be done in Python -->
   <Field Name="BMP4"> </Field>
   <Field Name="SHH"> </Field>
   <Field Name="WNT"> </Field>
   <!-- <Field Name="TOXIC_FLUID"> </Field> -->
      <!-- <Secretion Type="ectoderm_L">0</Secretion> -->
      <!-- <Secretion Type="ectoderm_R">0</Secretion> -->

</Plugin>

<Steppable Type="DiffusionSolverFE">

    <DiffusionField Name="BMP4">
      <DiffusionData>
         <FieldName>BMP4</FieldName>
         <GlobalDiffusionConstant>3.6</GlobalDiffusionConstant> <!-- Was 0.2 -->
         <GlobalDecayConstant>0.00050</GlobalDecayConstant> <!-- Was 0.0010 -->
         <DiffusionCoefficient CellType="wall">0.0</DiffusionCoefficient>
         <DecayCoefficient CellType="Medium">0.00350</DecayCoefficient> <!-- Was 0.0050 -->
         <DecayCoefficient CellType="wall">1</DecayCoefficient>
      </DiffusionData>
    </DiffusionField>

    <DiffusionField Name="SHH">
      <DiffusionData>
         <FieldName>SHH</FieldName>
         <GlobalDiffusionConstant>3.6</GlobalDiffusionConstant> <!-- Was 6.7 -->
         <GlobalDecayConstant>0.00050</GlobalDecayConstant> <!-- Was 0.00050 -->
         <DiffusionCoefficient CellType="wall">2.5</DiffusionCoefficient>
         <DecayCoefficient CellType="Medium">0.00350</DecayCoefficient>
         <DecayCoefficient CellType="wall">1</DecayCoefficient>

      </DiffusionData>
    </DiffusionField>

    <DiffusionField Name="WNT">
      <DiffusionData>
         <FieldName>WNT</FieldName>
         <GlobalDiffusionConstant>3.6</GlobalDiffusionConstant> <!-- Was 0.2 -->

         <GlobalDecayConstant>0.00050</GlobalDecayConstant>
         <DecayCoefficient CellType="Medium">0.00350</DecayCoefficient>
         <DiffusionCoefficient CellType="wall">0</DiffusionCoefficient>
      </DiffusionData>
    </DiffusionField>

    <DiffusionField Name="NOGGIN">
```

```
        <DiffusionData>
            <FieldName>NOGGIN</FieldName>
            <GlobalDiffusionConstant>3.6</GlobalDiffusionConstant> <!-- Was 0.2 -->
            <GlobalDecayConstant>0.00050</GlobalDecayConstant>
            <DiffusionCoefficient CellType="wall">3.6</DiffusionCoefficient>
            <DecayCoefficient CellType="Medium">0.00350</DecayCoefficient> <!-- Was 0.0050 -->
            <DecayCoefficient CellType="wall">1</DecayCoefficient>

        </DiffusionData>
      </DiffusionField>

        <BoundaryConditions>
            <Plane Axis="X">
                <!-- <ConstantValue PlanePosition="Min" Value="100.0"/> -->
                <!-- <ConstantValue PlanePosition="Max" Value="0"/> -->
                <!-- Other options are (examples): -->
                <Periodic/>
                <!-- <ConstantDerivative PlanePosition="Min" Value="10.0"/> -->
            </Plane>
            <Plane Axis="Y">
                <!-- <ConstantDerivative PlanePosition="Min" Value="100.0"/> -->
                <!-- <ConstantDerivative PlanePosition="Max" Value="0"/> -->
                <!-- Other options are (examples): -->
                <Periodic/>
                <!-- <ConstantValue PlanePosition="Min" Value="10.0"/> -->
            </Plane>
        </BoundaryConditions>
    </Steppable>



    <!-- <Plugin Name="LengthConstraintLocalFlex"> -->

        <!-- &lt;!&ndash; Applies elongation constraint to each cell. Users specify the length
        major axis -TargetLength and a strength of the constraint -LambdaLength. Parameters are
        specified for each cell individually &ndash;&gt;  -->


        <!-- &lt;!&ndash; IMPORTANT: To prevent cell fragmentation for large elongations you need
        to also use connectivity constraint &ndash;&gt;  -->


        <!-- This plugin currently works only in 2D. Use the following Python syntax to set/modify
        length constraint: -->
        <!-- self.lengthConstraintFlexPlugin.setLengthConstraintData(cell,20,30)  # cell ,
        lambdaLength, targetLength   -->
    <!-- </Plugin> -->

    <Plugin Name="OrientedGrowth">
         <Penalty>150</Penalty>
         <Falloff>5</Falloff>
    </Plugin>

     <Plugin Name="ExternalPotential">
     </Plugin>

     <Plugin Name="LengthConstraint">
         <LengthEnergyParameters CellType="endoderm" TargetLength="14" LambdaLength="10"/>
     </Plugin>

     <!-- <Steppable Type="PIFInitializer"> -->
```

```
        <!-- <PIFName>Simulation/initial_layout.piff</PIFName> -->
    <!-- </Steppable> -->

   <!-- <Steppable Type="UniformInitializer"> -->

        <!-- &lt;!&ndash; &lt;!&ndash; fluid &ndash;&gt;  &ndash;&gt;  -->
        <!-- &lt;!&ndash; <Region> &ndash;&gt;  -->
            <!-- &lt;!&ndash; <BoxMin x="0" y="50" z="0"/> &ndash;&gt;  -->
            <!-- &lt;!&ndash; <BoxMax x="210" y="100" z="1"/> &ndash;&gt;  -->
            <!-- &lt;!&ndash; <Gap>0</Gap> &ndash;&gt;  -->
            <!-- &lt;!&ndash; <Width>7</Width> &ndash;&gt;  -->
            <!-- &lt;!&ndash; <Types>fluid</Types> &ndash;&gt;  -->
        <!-- &lt;!&ndash; </Region> &ndash;&gt;  -->



       <!-- &lt;!&ndash; ENDODERM &ndash;&gt;  -->


        <!-- <Region> -->
            <!-- <BoxMin x="0" y="36" z="0"/> -->
            <!-- <BoxMax x="210" y="43" z="1"/> -->
            <!-- <Gap>0</Gap> -->
            <!-- <Width>10</Width> -->
            <!-- <Types>endoderm</Types> -->
        <!-- </Region> -->




        <!-- &lt;!&ndash; &lt;!&ndash; somite LEFT &ndash;&gt;  &ndash;&gt;  -->




        <!-- &lt;!&ndash; <Region> &ndash;&gt;  -->


            <!-- &lt;!&ndash; <BoxMin x="0" y="50" z="0"/> &ndash;&gt;  -->


            <!-- &lt;!&ndash; <BoxMax x="84" y="78" z="1"/> &ndash;&gt;  -->


            <!-- &lt;!&ndash; <Gap>0</Gap> &ndash;&gt;  -->


            <!-- &lt;!&ndash; <Width>7</Width>  &ndash;&gt;  -->


            <!-- &lt;!&ndash; <Types>mesoderm</Types> &ndash;&gt;  -->


        <!-- &lt;!&ndash; </Region> &ndash;&gt;  -->

        <!-- &lt;!&ndash; somite RIGHT &ndash;&gt;  -->


        <!-- <Region> -->
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```

```
            <!-- <Gap>0</Gap> -->
            <!-- <Width>7</Width> -->
            <!-- <Types>mesoderm</Types> -->
        <!-- </Region> -->

                <!-- &lt;!&ndash; ECM &ndash;&gt;  -->
        <!-- <Region> -->
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            <!-- <BoxMax x="126" y="57" z="1"/> -->
            <!-- <Gap>0</Gap> -->
            <!-- <Width>7</Width> -->
            <!-- <Types>ECM</Types> -->
        <!-- </Region> -->

        <!-- &lt;!&ndash; <Region> &ndash;&gt;  -->
            <!-- &lt;!&ndash; <BoxMin x="126" y="50" z="0"/> &ndash;&gt;  -->
            <!-- &lt;!&ndash; <BoxMax x="133" y="57" z="1"/> &ndash;&gt;  -->
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            <!-- &lt;!&ndash; <Types>ECM</Types> &ndash;&gt;  -->
        <!-- &lt;!&ndash; </Region> &ndash;&gt;  -->

        <!-- &lt;!&ndash; NOTOCHORD &ndash;&gt;  -->
        <!-- <Region> -->
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            <!-- <BoxMax x="126" y="51" z="1"/> -->
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            <!-- <Width>1</Width>  -->
            <!-- <Types>notochord</Types> -->
        <!-- </Region> -->

        <!-- <Region> -->
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            <!-- <Width>1</Width>  -->
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        <!-- <Region> -->
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            <!-- <Width>1</Width>  -->
            <!-- <Types>notochord</Types> -->
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        <!-- <Region> -->
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            <!-- <Width>1</Width>  -->
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        <!-- <Region> -->
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            <!-- <BoxMax x="120" y="54" z="1"/> -->
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            <!-- <Width>1</Width>  -->
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```

```
        <!-- </Region> -->

        <!-- <Region> -->
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        <!-- <Region> -->
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            <!-- <Width>1</Width>  -->
            <!-- <Types>notochord</Types> -->
        <!-- </Region> -->

        <!-- &lt;!&ndash; <Region> &ndash;&gt;  -->
            <!-- &lt;!&ndash; <BoxMin x="86" y="47" z="0"/> &ndash;&gt;  -->
            <!-- &lt;!&ndash; <BoxMax x="123" y="53" z="1"/> &ndash;&gt;  -->
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            <!-- &lt;!&ndash; <Width>1</Width>  &ndash;&gt;  -->
            <!-- &lt;!&ndash; <Types>notochord</Types> &ndash;&gt;  -->
        <!-- &lt;!&ndash; </Region> &ndash;&gt;  -->


        <!-- &lt;!&ndash; <Region> &ndash;&gt;  -->
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            <!-- &lt;!&ndash; <BoxMax x="126" y="57" z="1"/> &ndash;&gt;  -->
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            <!-- &lt;!&ndash; <Width>7</Width>  &ndash;&gt;  -->
            <!-- &lt;!&ndash; <Types>notochord</Types> &ndash;&gt;  -->
        <!-- &lt;!&ndash; </Region> &ndash;&gt;  -->







        <!-- &lt;!&ndash; NeuralPlate_L &ndash;&gt;  -->


        <!-- <Region> -->
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            <!-- <Width>7</Width> -->
            <!-- <Types>np_basal_R</Types> -->
        <!-- </Region> -->

        <!-- <Region> -->
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            <!-- <Width>7</Width> -->
            <!-- <Types>np_lateral_R</Types> -->
        <!-- </Region> -->

        <!-- <Region> -->
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```

```
        <!-- <Gap>0</Gap> -->
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        <!-- <Types>np_apical_R</Types> -->
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    <!-- <Region> -->
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        <!-- <Width>7</Width> -->
        <!-- <Types>np_basal_R</Types> -->
    <!-- </Region> -->

    <!-- <Region> -->
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        <!-- <BoxMax x="56" y="78" z="1"/> -->
        <!-- <Gap>0</Gap> -->
        <!-- <Width>7</Width> -->
        <!-- <Types>np_lateral_R</Types> -->
    <!-- </Region> -->

    <!-- <Region> -->
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        <!-- <Types>np_apical_R</Types> -->
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    <!-- &lt;!&ndash; <Region> &ndash;&gt;  -->
        <!-- &lt;!&ndash; <BoxMin x="21" y="71" z="0"/> &ndash;&gt;  -->
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        <!-- &lt;!&ndash; <Gap>0</Gap> &ndash;&gt;  -->
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    <!-- &lt;!&ndash; <Region> &ndash;&gt;  -->
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    <!-- &lt;!&ndash; </Region> &ndash;&gt;  -->

    <!-- &lt;!&ndash; <Region> &ndash;&gt;  -->
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        <!-- &lt;!&ndash; <Width>7</Width> &ndash;&gt;  -->
        <!-- &lt;!&ndash; <Types>np_apical_R</Types> &ndash;&gt;  -->
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    <!-- &lt;!&ndash; NeuralPlate_R &ndash;&gt;  -->

    <!-- <Region> -->
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        <!-- <Gap>0</Gap> -->
        <!-- <Width>7</Width> -->
```

```
        <!-- <Types>np_basal_R</Types> -->
    <!-- </Region> -->

    <!-- <Region> -->
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        <!-- <BoxMax x="154" y="71" z="1"/> -->
        <!-- <Gap>0</Gap> -->
        <!-- <Width>7</Width> -->
        <!-- <Types>np_lateral_R</Types> -->
    <!-- </Region> -->

    <!-- <Region> -->
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        <!-- <Width>7</Width> -->
        <!-- <Types>np_apical_R</Types> -->
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    <!-- <Region> -->
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        <!-- <Width>7</Width> -->
        <!-- <Types>np_basal_R</Types> -->
    <!-- </Region> -->

    <!-- <Region> -->
        <!-- <BoxMin x="154" y="71" z="0"/> -->
        <!-- <BoxMax x="189" y="78" z="1"/> -->
        <!-- <Gap>0</Gap> -->
        <!-- <Width>7</Width> -->
        <!-- <Types>np_lateral_R</Types> -->
    <!-- </Region> -->

    <!-- <Region> -->
        <!-- <BoxMin x="154" y="78" z="0"/> -->
        <!-- <BoxMax x="189" y="85" z="1"/> -->
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        <!-- <Width>7</Width> -->
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        <!-- &lt;!&ndash; MHP &ndash;&gt;  -->


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        <!-- &lt;!&ndash; ECTODERM &ndash;&gt;  -->




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        <!-- <Region> -->
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        <!-- &lt;!&ndash; <Region> &ndash;&gt;  -->
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```
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            <!-- &lt;!&ndash; <Types>fluid</Types> &ndash;&gt;  -->
        <!-- &lt;!&ndash; </Region> &ndash;&gt;  -->



    <!-- </Steppable> -->
</CompuCell3D>
```

```
## Data Output Parameters ##
# Directory where simulation output data will be saved in the form of a dictionary (set to a
suitable location)
cell_data_dir = r"C:\Users\jobbe\PUBLICATION_DATA\Nature_version\Control\celldata"
# Frequency (in MCS steps) at which cell information will be saved
save_cell_info_frequency = 100

## Cell Initialization Parameters ##
# Notochord dimensions and position
notochord_radius_x = 21  # Radius of the notochord in the x-axis
notochord_radius_y = 7   # Radius of the notochord in the y-axis
notochord_xCOM = 0.5     # X center of mass for notochord positioning
notochord_yCOM = 0.20    # Y center of mass for notochord positioning

# Neural epithelium (NE) cell dimensions
ne_cell_width = 7
ne_cell_height = 7

# Surface ectoderm (SE) cell dimensions and number
se_cell_width = 7
se_cell_height = 7
se_cell_number = 3  # Number of cells in the SE layer

# Neural crest (NC) cell count
nc_cell_number = 1       # Number of NC cells in the x direction
nc_cell_number_y = 3     # Number of NC cells in the y direction

# Endoderm cell dimensions
endoderm_cell_width = 14
endoderm_cell_height = 5

## General Cell Parameters ##
standard_target_volume = 49          # Initial target volume (based on 7x7 cell layout)
standard_lambda_volume = 2.0         # Volume constraint factor (lambda)
cell_cycle_time = 4000               # Number of MCS per cell cycle
mesoderm_growth_rate = 0.02          # Volume increment per MCS in mesoderm
mesoderm_max_growth_rate = 0.1       # Maximum mesoderm growth rate (for hill function)
start_somitogenesis = 4000           # Time (in MCS) to start somitogenesis
ectoderm_growth_rate = 0.0030        # Volume increment per MCS in ectoderm
neuralcrest_growth_rate = 0.0030     # Volume increment per MCS in neural crest
somite_growth_rate = 0.0010          # Volume increment per MCS in somite
ECM_growth_rate = 0.0050             # Volume increment per MCS in ECM
somite_formation_prob = 1/2500       # Probability for somite formation
apoptosis_chance = 1/10000           # Probability for a cell to undergo apoptosis

## Cell Behavior Parameters ##
# Apical constriction parameters
enable_apical_constriction_behavior = True      # Enable apical constriction
enable_mhp_formation = True                     # Enable medial hinge point (MHP) formation
apical_constrict_target_volume = 21             # Min target volume for apical cells
basal_constrict_target_volume = 77              # Max target volume for basal cells
mhp_constrict_frequency = 1                     # Frequency of MHP constriction (in MCS)
apical_constrict_frequency = 1                  # Frequency of apical constriction steps
delta_target_volume = 0.04                      # Change in target volume per step
delta_lambda_volume = 0.008                     # Lambda volume change per step
delta_target_volume_apical = 0.04               # Apical volume change per step
apical_constriction_elongation = 18             # Target FPP link distance for constricted cells
delta_elongation = 0.01                         # Elongation change per step
standard_elongation = 12                        # Standard FPP link distance for cells

# Midline migration forces for neural crest and ectoderm cells
```

```
nc_midline_migration_force_low = 1.00
nc_midline_migration_force_high = 1.05
ec_midline_migration_force_low = 1.00
ec_midline_migration_force_high = 1.05

## Gradient Parameters ##
# Parameters for various molecular gradients (BMP4, SHH, ATRA, FGF, WNT, NOG)
BMP4_secretion_constant = 0.0150
BMP4_FGF_inhibition = 0.0150

NC_SHH_secretion_constant = 0.0070
NC_FGF_induction = 0.0070
FP_SHH_secretion_constant = 0.0070
FP_SHH_induction = 0.0070

ATRA_secretion_constant = 0.0035
FGF_secretion_constant = 0.0035

WNT_secretion_constant = 0.0070
WNT_ATRA_FGF_induction = 0.0070

NOG_secretion_constant = 0.00025
NOG_induction = 0.0060

# Dictionary defining expression levels for various genes in each cell
gene_status_dict = {
    "BMP4": 1.0,
    "SHH": 1.0,
    "WNT": 1.0,
    "ATRA": 1.0,
    "FGF": 1.0,
    "NOG": 1.0,
    "ZIC": 1.0,
    "PAX3": 1.0,
    "GLI2": 1.0,
    "CHD1": 1.0,
    "SNAI1": 1.0,
    "PTCH1_r": 1.0,
    "BMPR_r": 1.0,
    "FGR1_r": 1.0,
    "FZD1_r": 1.0
}

## Toxicological Perturbation Parameters ##
dose_toxic_substance = False  # Set to True to induce a toxic field affecting NE cells

## Apical Constriction FPP Links Parameters ##
# Initial and apical constriction values for FPP (Fixed Point Particles) link distances
initial_lambda_distance = 20.0
initial_target_distance = 5.0
initial_max_distance = 20.0
apical_constrict_lambda_distance = 40.0
apical_constrict_target_distance = 0.0
basal_constrict_target_distance = 9.0
apical_constrict_max_distance = 15.0
delta_distance = 0.005  # Step change in FPP link distance

## Cell Layout Parameters ##
use_ECM = True  # Initialize an extra ECM layer around mesodermal cells
```