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Project acronym: ONTOX

Project title: ontology-driven and artificial intelligence-based repeated dose toxicity testing of chemicals for next generation risk assessment

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Work package:	WP9
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Contents

1. Introduction and objectives

The ONTOX project aims to deliver a generic strategy to create innovative new approach methodologies (NAMs) in order to predict systemic repeated dose toxicity effects for human safety assessment. For proof-of-concept purposes, Three organ systems have been selected, namely the liver (WP7), the kidney (WP8), and the developing brain (WP9). Within WP9, a computational model in CompuCell3D software (www.compucell3D.org)[1] that visualizes neural tube closure and can be challenged by changes in gene expression and cell characteristics, which mimic the effects of chemicals observed in dedicated in vitro assays will be developed.
This deliverable shows the current progress of our computational model that recapitulates the morphogenetic events of mammalian neural tube closure. The model is based on a physiological map of mammalian neural tube closure we developed earlier[2] and an additional literature search. Parameter variation experiments were performed to test the accuracy of the model. In these experiments, the model predicted structural and dynamic phenotypes reminiscent of neural tube defects seen in humans and other mammals. A research article that thoroughly describes this work is currently in development.

2. Results

2.1. A computational model of mammalian neural tube closure

The model of mammalian neural tube closure was built using CompuCell3D, an open-source platform that simulates multicellular systems. This platform takes into account the specific behaviors of cells, their regulatory signals, and physical properties[1]. The two-dimensional model represents a dorso-ventral section of the middle spinal region of the neural tube (Figure 1). Within this region, both median hinge points as well as dorsolateral hinge points are present during neural tube closure[3].

The model starts as a neural plate, measuring 252µm by 50µm by 1µm, and over 50 hours, it undergoes the morphogenetic changes necessary to form a closed neural tube [4]. The start of the model approximates gestational day 28, Carnegie Stage 10 in human development. To make the model as human-like as possible, its physical properties, time constraints and spatial

organization were adjusted to cover the key characteristics of human neural tube closure[4]. The model displays key morphogenetic events similar to those seen in reconstructed sections of the human embryo and mouse embryo[4]. These events are directly driven by a complex gene regulatory network (figure 2).

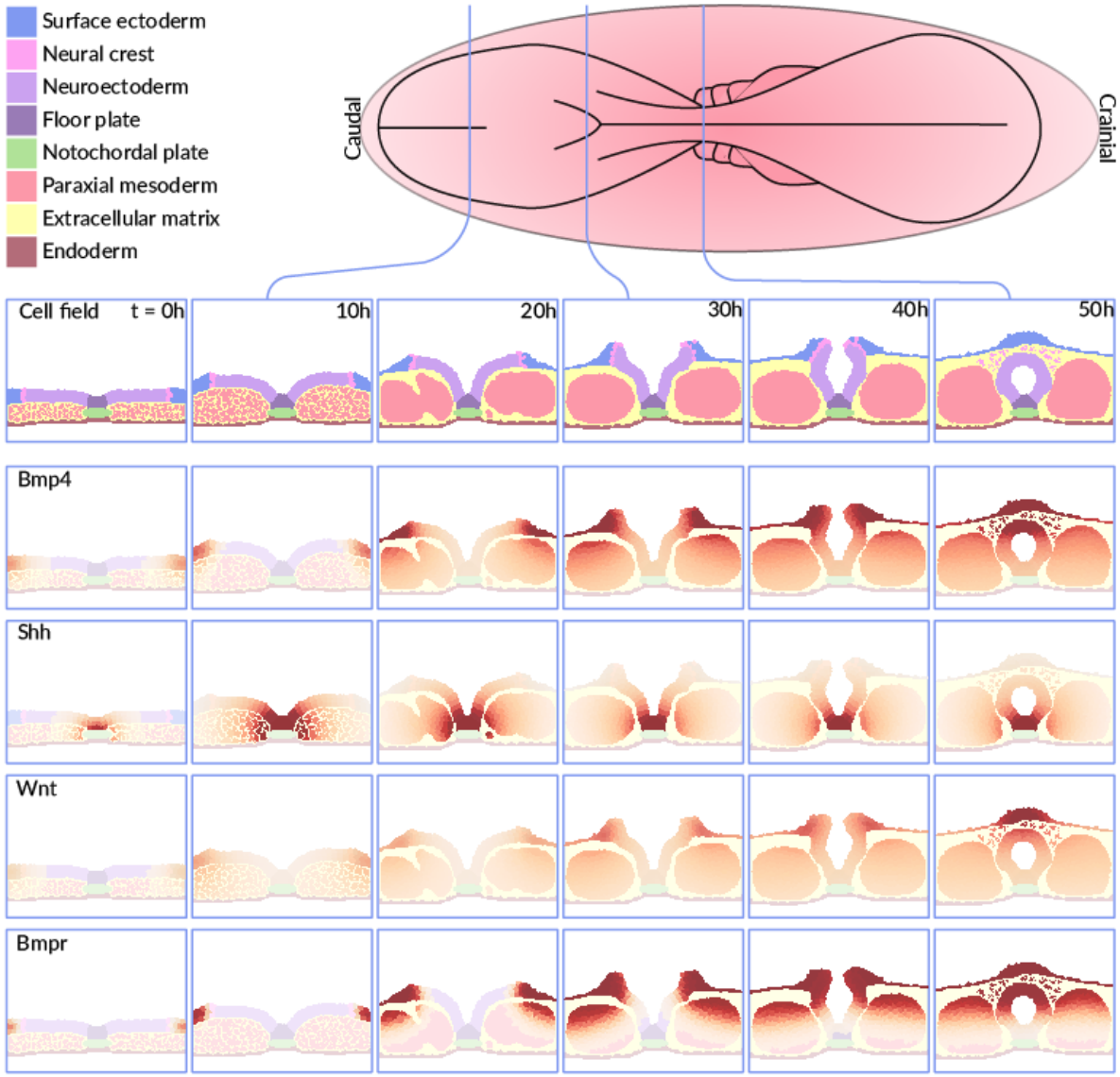

Figure 1: A computational model of mammalian neural tube closure. The 8 different cell types featured in the model are indicated in legend with their respective color. Relative gene expression of 4 arbitrarily chosen key genes is visualized, with gene names indicated in the top left corner of the first image of the series. The darkest color represents the highest gene expression and white the lowest. As indicated by the schematic, the model represents a dorso-ventral section of the mid-spinal region of the embryo. At t=0h the size of the model is 252µm by 50µm by 1µm.

2.2. The gene regulatory network that drives key processes of the neural tube closure

To simulate the complex interplay of various genes and proteins during neural tube closure in

the model, a gene regulatory network of neural tube closure was created, containing key genes[2]. The network regulates three key processes for successful neural tube closure. These are floor plate formation, dorsolateral hinge point formation and neural crest cell delamination. The network primarily relies on animal data because mechanistic data of the human neural tube is relatively scarce. This reliance could lead to unforeseen irregularities, even though neural tube closure is highly conserved among vertebrates[2]. The network employs Hill functions to approximate the binding of proteins to receptors and activation or inhibition of gene transcription. The Hill functions in the network are scaled between 0 and 1 with a Hill coefficient (n) of 8 and a half-maximal constant (K) of 0.5, due to the absence of precise parameter values in the available data. To acknowledge the inherent variability and unpredictability in biological systems, each gene in the network contains a random base transcription level.

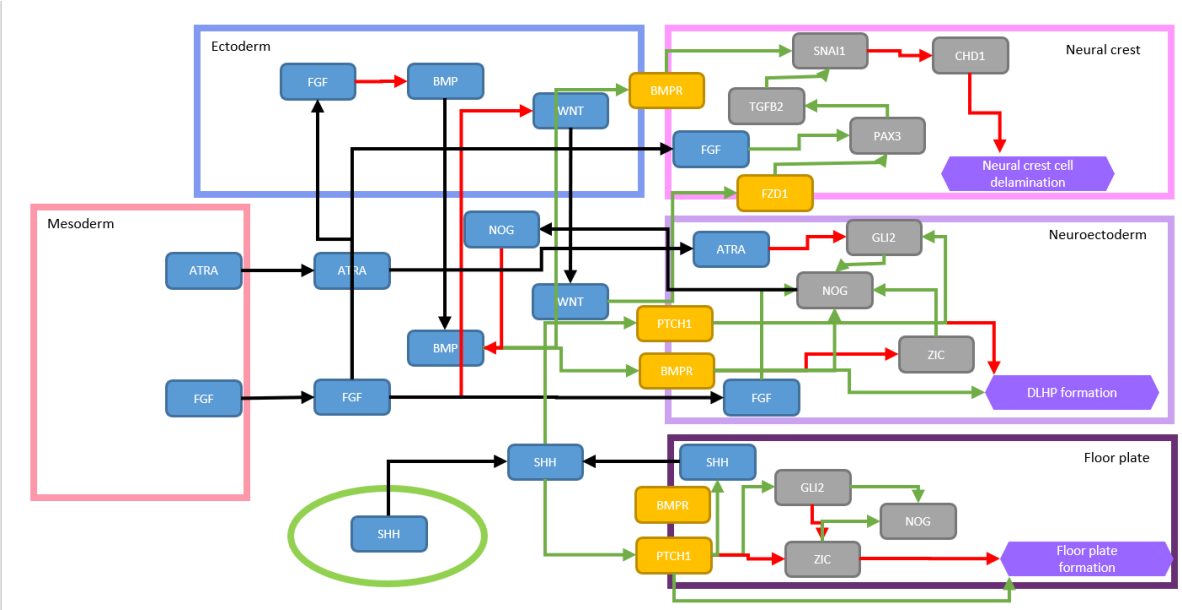

Figure 2: Gene regulatory network driving the neural tube closure model. The network regulates three key processes for successful neural tube closure (purple diamonds). These are Floor plate formation, dorsolateral hinge point formation and neural crest cell delamination. Yellow boxes represent receptors. Blue boxes protein gradients and grey boxes intracellular genes. The black arrows indicate transport, the red arrows indicate inhibition and the green arrows promotion.

2.3. Towards modeling neural tube closure defects

To validate the computational model against known biological data and explore potential developmental anomalies, *in silico* parameter variation experiments were performed (Figure 3). In these experiments, 16 perturbation scenarios were tested. 8 different genes that are involved in the regulation of the three key processes (floor plate formation, DLHPs formation, neural crest cell delamination) were hyperactivated (x2 fold change) or knocked out. In the perturbation scenarios, the model revealed neural tube closure defects consistent with the literature and specific structural phenotypes seen in humans and other mammals [5–18].

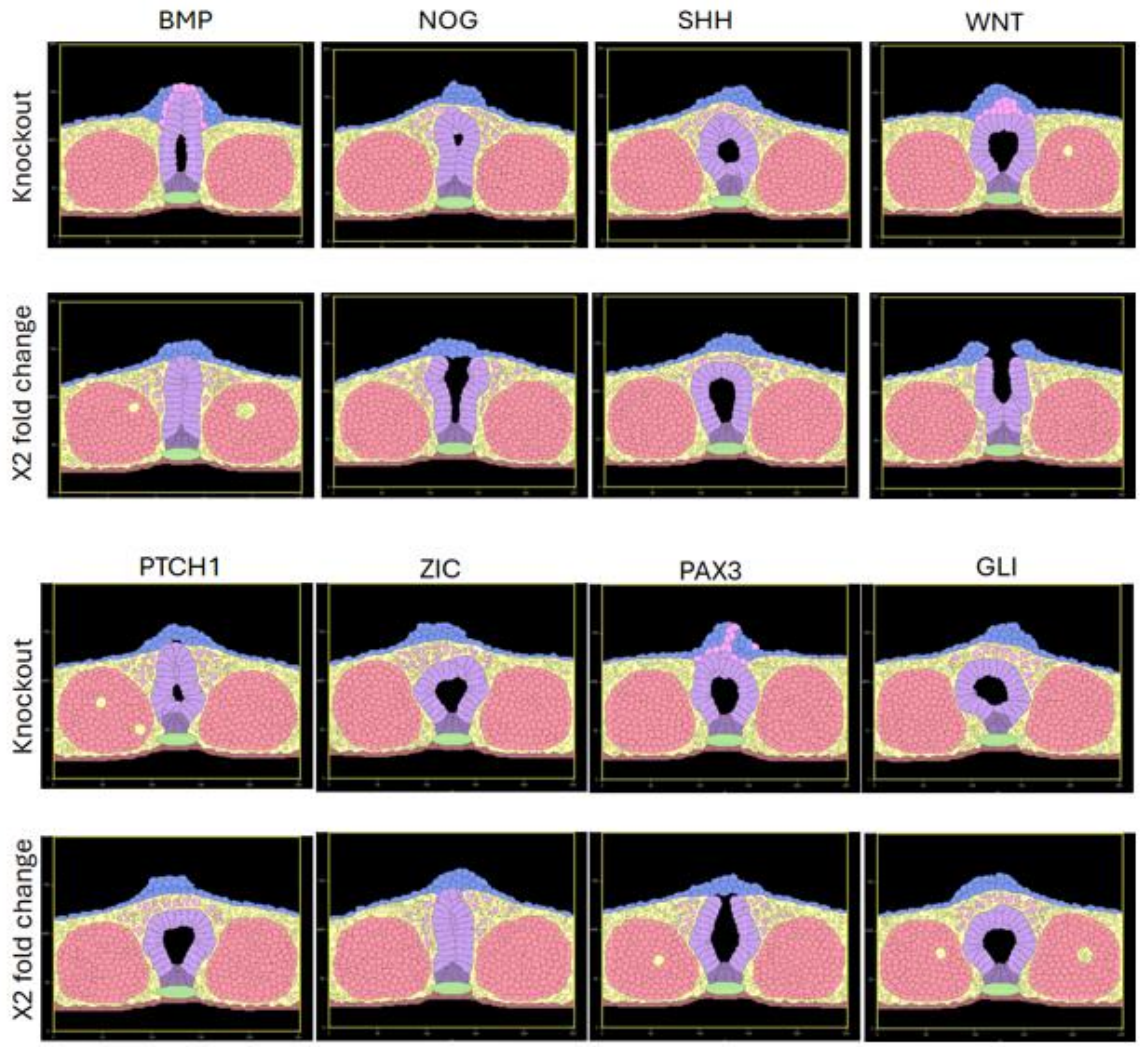

Figure 3: Towards modeling neural tube closure defects. The gene that is perturbed is noted at the top. The perturbation scenario (gene knockout or x2 fold change) is indicated at the left. The 8 different cell types featured in the model and their respective colors are presented in the color legend in figure 1. The control scenario (without perturbations) is presented in Figure 1. The extracellular matrix can be captured within the somite/paraxial mesoderm randomly, which is not a result of the perturbation scenario.

3. Conclusions and follow-up

In conclusion, task 9.3 was successfully carried out, delivering an optimized computational model of mammalian neural tube closure, which was applied to predict neural tube defects caused by genetic perturbations. The model generated during the current study is available in the BioStudies database (http://www.ebi.ac.uk/biostudies) under accession numbers S-ONTX34. A research article that thoroughly describes this work is currently in development. Future applications of the model include studying the effect of chemical related perturbations on neural tube closure. Changes in gene expression caused by chemical exposure observed in dedicated *in vitro* assays will be replicated as adjusted parameters in our model. This allows to

study chemical-related effects on neural tube closure based on *in vitro* assay results integrated in an *in silico* model covering the relevant mechanistic biology.

4. Delays, issues and contingency

As reported earlier, the current state of technical development of the software platform used, does not allow the development of a 3D model of a process as complex as neural tube closure that would be useful for the prediction of chemical/genetically induced neural tube defects. Therefore, the study focused on a 2D section of the neural tube and modeling the development over time. Even though this approach overlooks a number of aspects of neural tube closure along the rostral-caudal axis (e.g. elongation, anterior-posterior patterning, and torsion), modification of the current 2D model will allow for capturing various locations across the tube, from the lower spine to the brain. Thus, the shortcomings of the 2D model can be compensated.

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