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Morphogenesis:
Bridging Theory and Simulation in Vertebrates

Genetic mechanisms of early embryonic development in vertebrates and insights into a novel modelling approach based on smoothed-particle hydrodynamics and GPU-programming

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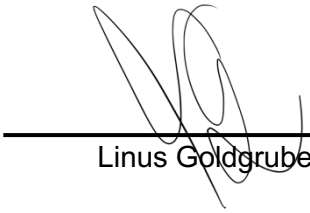
DECLARATION

Herby, I declare, that this thesis was solely written independently and has not been published elsewhere, in part or as a whole. References and acknowledgements regarding citations are provided and summarised in an attached list.

Vienna, July 26th, 2024



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ABSTRACT

Morphogenesis can be defined as the process by which living organisms build up a functional anatomical body plan. The concept dates back to Aristotle, but the first empirical studies began in the late 19th century with observations of embryogenesis. Morphogenesis has been applied to various scales, yet many of the highly complex and intricate underlying realisations and levels of emergence still need to be explored.

Over the last decades, great progress has been made in the field of developmental biology. In addition to the identification of numerous morphogens and effector molecules and their interactions, new research methods and tools have been developed, and mathematical and code-based frameworks have become more practical. This led to a bottom-up and top-down approach to morphogenesis. Optogenetics, but also organoids, gene editing and bioelectrical recordings and manipulations, have contributed much to bottom-up research by increasing spatial and temporal resolution and providing unprecedented details of the underlying molecular mechanisms. The application of computational models, spatial statistics, information theory and dynamical systems theory, is responsible for much of the rise of top-down approaches in morphogenesis, but also in various other fields such as regeneration, oncology, or psychology, to name a few. Converging bottom-up and top-down approaches will enable faster and more accurate progress in morphogenesis.

This thesis provides an overview of the current state of research and tries to establish a link between practical, computational approaches and neurodevelopmental theory. Starting with the zygote, it leads through to the structural and functional formation of the earliest, most important morphogenetic processes. It focuses on the underlying principles and applicable models of pattern formation of key tissues and organs. It touches on the self-organising interactions between the nervous system, the uterine environment, and the later-forming body as fundamental properties of the body are dependent on these interactions to develop properly. The discussed mathematical approaches and a simulation program based on smoothed particle hydrodynamics (SPH), a modelling approach of particular interest, are presented and integrated into the modelling landscape of tissue mechanics. Three practical examples of morphogenetic processes are provided as Python scripts that could serve as a stepping stone in transferring the known theory into the modelling program and could help to gain a better understanding of the goal of modelling embryonic development.

ABBREVIATIONS

TDC	Tissue Derived Cell
iPSC	Induced Pluripotent Stem Cell
CNS	Central Nervous System
PNS	Peripheral Nervous System
GUI	Graphical User Interface
IDE	Integrated Development Environment
NVCC	Nvidia CUDA Compiler
MPI	Message Passing Interface

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1 INTRODUCTION

1.1 Morphogenesis

Specialised work is regarded as a key component in the formation of advanced civilisations. The same holds for the emergence of multicellular life. The idea of morphogenesis, or generation of shape, dates back to Aristotle and other Greek philosophers, pre-dating many biological principles by centuries. Morphogenesis is generally defined as a **developmental, pattern-forming process that dynamically regulates functional body plans**. There is no one right way to define the underlying phenomenon that can be applied to many layers of emergence. We can distinguish between **intracellular** (e. g. cytoskeletal turnover or the formation of the mitotic spindle through polymerisation of microtubules, ...), **intercellular** (e. g. communication via local diffusion of morphogens or programmed cell death over the extrinsic pathway, …), **inter-organ** (e. g. gut-brain axis, heart-lung interactions, …) and **inter-organismal** interactions (e. g. verbal communication, cooperation in packs, governments…). This demonstrates that the fundamental mechanisms of morphogenesis and the results of its study have far-reaching implications in many fields. Naturally, implications of research on this topic can lead to developments in reducing or treating congenital defects and other developmental abnormalities, as well as enable biomedicine to tackle issues of oncology and regeneration. Besides these more obvious biomedical implications, due to its fundamental importance in life, it could also lead to unexpected and impactful results outside the biological domain such as psychology or robotics.

1.1.1 General Overview

Morphogenesis and developmental biology as a field properly originated at the end of the 19th century in a concept called **Entwicklungsmechanik** (Eng. “Development mechanics”), a precursor to developmental physiology. Driesch (1891) made some first important observations about the functional interdependence of shape generation between the level of the why and its sub-components. Although it was not called “morphogen” at that time, the first designation of morphogens as following **gradients** was put forth by Morgan (1897, 1901). He speculated about the existence of a “formative substance” that governs the regenerative process of worms, depending on position. Boveri (1901) expanded on this notion and thought of this form-providing substance as patterning the whole developing organism via a multitude of gradients. The proof of a highly speculated localised “organising centre” first appeared with

the discovery of the **Spemann-Mangold-Organiser** (Spemann & Mangold, 1924) which established the dorsoventral and anteroposterior axes in amphibian embryos, but similar regions were later discovered in other groups of animals.

In 1938, Albert Dalcq and his PhD student Jean Pasteel proposed for the first time, that these formative substances can **diffuse** through tissue, following experiments with different types of mesoderm. In their discussion, they note that by placing somatic and splanchnic mesoderm next to each other, the initial difference in concentration leads to establishing a medium concentration in the centre from which the corresponding tissue subsequently forms.

A period of exploration of different models followed, including the initial gradient concept by Gierer & Meinhardt (1972) and the analysis of morphological features of certain groups of animals, where Sander (1959) and many others (Hunt, 1975; Lawrence & Burden, 1973) performed seminal work that built a strong case for the morphogenetic process as a **dynamical, evolvable system**. This notion is often referred to when introducing the subject, as it is the central difference between life and machines. The latter are only expected to work once their construction has been completed, whereas living beings must function, i. e. keep a far-from-equilibrium steady-state, *while* developing.

The term morphogen was first introduced by **Turing** (1952) when he proposed his formalism of **reaction-diffusion**. He showed that the complexity of the interaction between two or more diffusing morphogens suffices to produce a range of patterns across a tissue, vividly illustrated by e. g. pigmentation of fish (Figure 1). The central extrapolation of this idea was performed by **Wolpert** with his famous **French Flag Problem** (1969). In his paper, he first explains size invariant pattern formation in tissue as a discretisation of a morphogen gradient via alterations of gene expression. Essentially, Turing's model explains how morphogen gradients can form from only stochastic differences, while Wolpert's model explains how cells use these gradients to make fate decisions. Although Turing patterns themselves are proportion-dependent, modified Turing patterns have been produced that are scale-invariant (Ishihara & Kaneko, 2006).

The **first morphogen identification** and systematic study on the genetic regulation of embryonic development was done by **Christiane Nüsslein-Vollhard** (Driever & Nüsslein-Volhard, 1988; Nüsslein-Volhard & Wieschaus, 1980). She identified a protein encoded by the *Bicoid* gene in the fruit fly Drosophila melanogaster, which plays a central role in the

anteroposterior patterning of the organism. She proved that morphogens establish gradients, contrary to popular belief at that time.

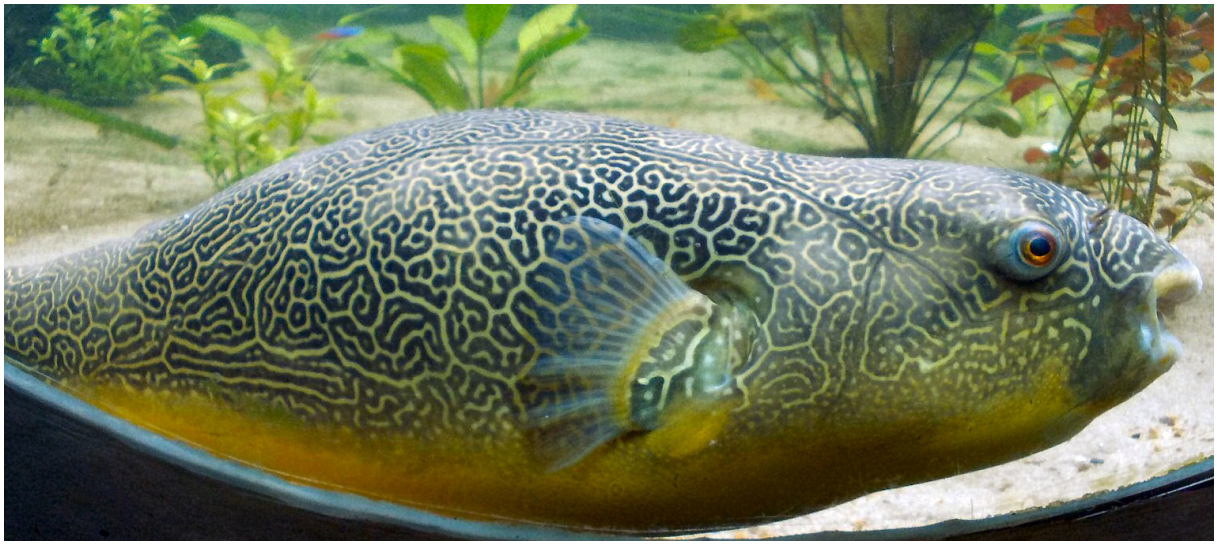

Figure 1: Turing patterns on the skin of a puffer fish. ⓒ

This illustrates the complex phenotypical appearance computable by a simple two-component reaction-diffusion system. Skin patterns like this appear in many species and appear in many different shapes and forms.

1.1.2 Origins of Morphogenesis in the Tree of Life

The **Ediacaran** is the earliest period known to host multicellular animals, the so-called Metazoans (Figure 2). It defines a span of 96 million years, up until the beginning of the Cambrian 538.8 Mya. Only recently in 2004, it became the newest addition to the list of geological periods, the first to be added in the last 120 years. Most notably, it contained a fast evolutionary radiation event known as the Avalon Explosion about 575.5 Mya. This led to the emergence of the first soft-bodied ancestral bilaterians and animals with other simple body symmetries, which mostly went extinct. The eumetazoans are usually divided into **Radiata** (Ctenophores and Cnidarians) and **Bilateria**, although multiple hypotheses still stand regarding the true phylogeny of the phyla of eumetazoans (Whelan et al., 2017). In the phylogenic tree, bilateral symmetry is assumed to have evolutionarily originated in the Ediacaran period and is a highly conserved trait in the early development of both protostomes and deuterostomes, which are defined by their first developing body opening being the mouth and the anus, respectively. Even echinoderms are bilateral as larvae, despite subsequently acquiring radial symmetry. The developmental origin of this bilateral symmetry in embryos

correlates with the asymmetry of the cytosol and the membrane proteins in the pronuclear zygote (Maienschein, 2016).

Cellular differentiation has already been present in unicellular life, but there are big differences in the cells' gene regulation and complexity of interaction (Sebé-Pedrós et al., 2017). **All known Eumetazoans** feature cooperating cell lineages with **invariant morphologies** and complex behaviour, well-defined body axes, and a **nervous system** (Feuda et al., 2017; Simion et al., 2017), which means that origin and evolutionary timing of both complex morphology and behaviour can only be hypothesised about (Jékely, 2021; Keijzer et al., 2013), but also suggests that one of those features affords others to emerge.

1.1.3 Understanding morphogenesis

Morphogenesis consists of several different mechanisms on a cellular level. These include chemical gradients, molecular clocks, lineage separation, cell taxis, adhesion, remodelling, and growth. A good textbook on this is provided by Davies (Davies, 2014). The tie between the genetic basis of morphogenesis and the mechanical mechanisms underlying the structural changes necessary for the establishment of the body plan remains unclear. A good overview of the most fundamental connections drawn is provided by Gilmour et al. (2017), also specifying the cellular mechanisms of tissue shaping. For more information about the most important pathways involved in cell differentiation and morphogenesis in general, see Albert Basson (2012).

Morphogenesis can be analysed from multiple conceptual perspectives. It can be understood as fundamentally being a **teleonomic** process (Levin, 2023)**.** Teleonomy can be briefly described as "evolved biological purposiveness" (Corning et al., 2023). Contrary to teleology, it offers a naturalistic explanation of purposiveness as a result of the evolutionary process. However, it should be mentioned that there is no consensus on how to define purpose to make it scientifically measurable, and agential thinking in some contexts is controversial. The concept could be regarded as part of the **Extended Synthesis Theory** (Müller, 2010; Pigliucci, 2007), an augmented framework of the paradigm of Modern Synthesis in evolutionary biology. In it, evolvability, phenotypical plasticity, epigenetics, complexity theory, and the theory of evolution in high dimensional adaptive landscapes are added to the current paradigm, **Modern Synthesis,** which was the reconciliation of Darwinian and Mendelian evolutionary accounts.

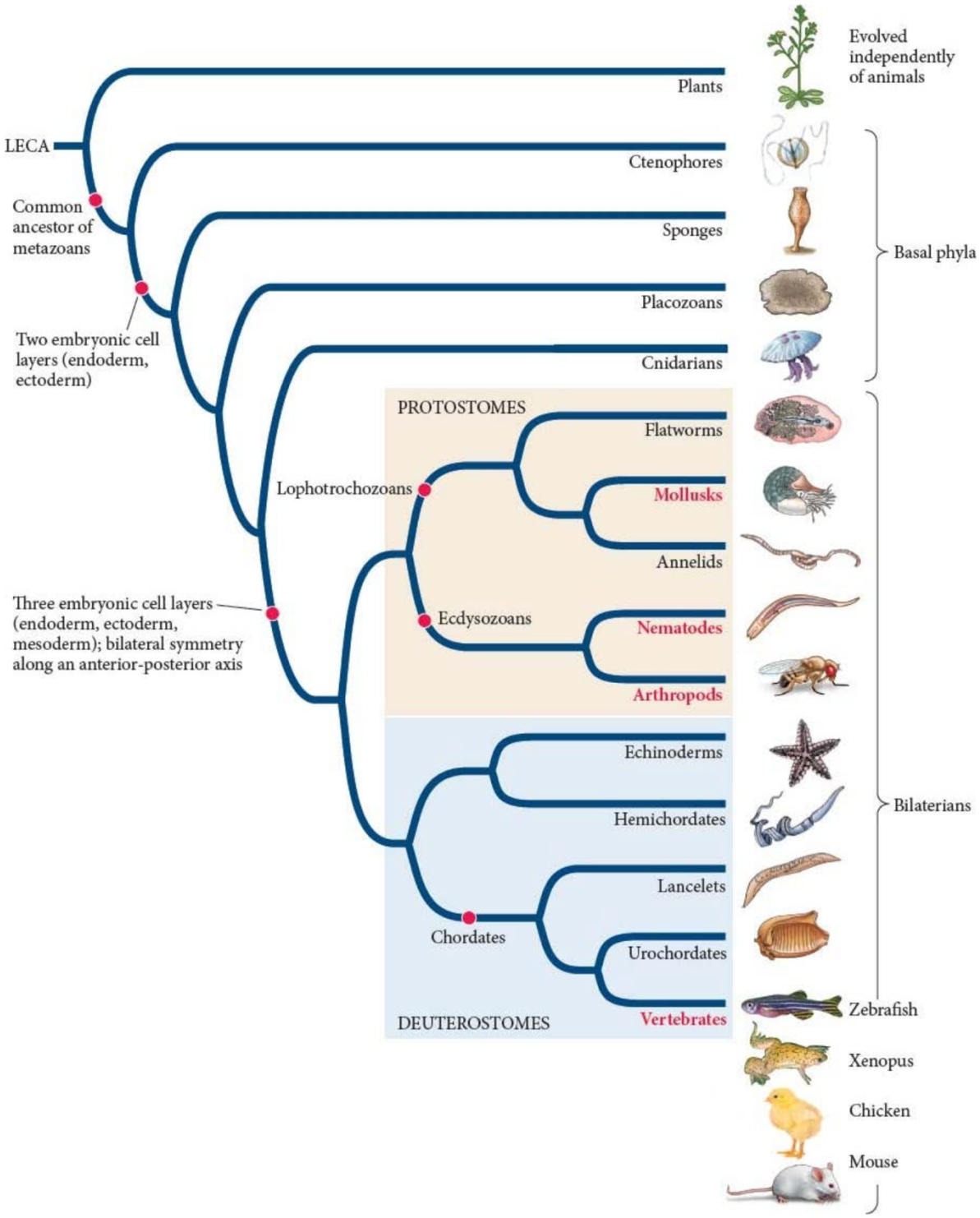

Figure 2: Stages of mouse and human preimplantation development, from (Gilbert SF., 2020).

Extended Synthesis Theory is controversial among biologists. Some researchers think of the Modern Synthesis to be sufficient for integrating heredity and formation of organisms. It is well-established at this point, however, that epigenetics, which is not part of the Modern Synthesis, plays an important role in evolutionary biology. This proves the Extended Synthesis to incorporate aspects that the Modern Synthesis is missing. Others argue that the Extended Synthesis" is not an expansion, but rather a frameshift, a necessary development away from population genetics. It cannot account for evolutionary means such as evolvability, emergence, and organisation. This was already apparent to Sewall Wright (1932), who knew that a realistic evolutionary biological model would require the modelling of combinatorial explosive possible gene combinations in a population. Developmental systems are a possible replacement for population genetics (Craig, 2010). Regardless of the theory, the common effort lies in the integration of heredity and form. This is also at the core of the Morphogenesis program (hereafter identifiable as written capitalised) which is the practical part of this thesis. Levin (2023) hypothesises the main function of the nervous system to be **morphological coordination**. He argues that the level of morphological complexity of animals would not be possible without some form of centralised, long-distance coordination of action. These self-organising interactions between the nervous system, the uterine environment, and the later-forming body are key for the body to develop properly. It is well-established that the organism outsources the acquisition of necessary information for the establishment of its body plan in the anatomical space. Many processes of realizing a body plan are interdependent on the simultaneous establishment of the CNS as pointed out by Levin and other sources (Mori & Kuniyoshi, 2010, 2012). An example of this would be the separation of adjacent muscle fibres and tendons in the developing limbs through the activation of independent groups of motor neurons by the developing CNS. The resulting shear forces lead to the detachment of the individual tendons and muscles from each other. A lack of foetal movement (or foetal akinesia) leads to diseases like Arthrogryposis, describing *multiple congenital contractures*.

Through the functional lens of dynamical systems theory, morphogenesis can be described as a **complex interaction between individual cells acting as far-from-equilibrium (FFE) steady-state systems trying to *minimise* variational free energy** (Ali et al., 2022; Hosoya et al., 2005). Briefly stated FFE or nonequilibrium processes are defined by the breaking of **time-reversal symmetry**. Maintenance of a steady-state as an FFE system is made possible by the constant exchange of energy and/or matter with the external world. This is closely related to the definition of self-organisation of systems, although not every FFE system is self-

organised. Cellular respiration or simple metabolic pathways might result from self-organisation, but these FFE steady-state systems maintaining homeostasis are not self-organising. According to Kuchling et al. (2020), applying **Bayesian inference** to the concept of variational free energy (VFE) can be helpful by viewing morphogenesis as a process of Bayesian belief updating. Bayesian inference is a statistical method that updates the probability of a hypothesis based on new evidence or data, combining prior knowledge with observed information. Individual cells compare their environment to their internal model and adjust accordingly. The

adjustment is also the process of minimising VFE, where cells move, grow, change their membrane receptor composition, change their junctions, etc. VFE is a quantity used to measure the difference between the true probability distribution of the data observed and the estimated probability distribution of the system. The true probability distribution is the real, almost always unknown, structural, and functional organisation of the environment, whereas the estimated probability distribution is the “knowledge” or interpretation of the probabilities as a probability model by the system. It expresses the fittedness of internal and active states to the environment. An illustrative example of this would be the true probability of a coin landing on either one of its sides. It is not exactly 50 %, as the coin could also land on its side. The probability model can be established experimentally and gives a model estimate of the situation at hand. This is also described as the **exploitation of emergence** by living organisms through the modelling of the environment (Halley & Winkler, 2008). Compared to organisms instinctively reacting to raw stimuli, putting sensory information into perspective, i. e. an emergent model, provides a better integration of the system into its environment. Crutchfield and Rohilla Shalizi view emergence as a measure of relative predictive efficiency (1999), which ties closely to minimising VFE. The first sentence of this paragraph can be paraphrased as follows: Morphogenesis is the result of cooperating cells that constantly adjust the internal model of their environment to approximate their role in the establishment and maintenance of the body plan.

1.1.4 Factors enabling the existence of complex morphologies?

There is evidence that the complex morphologies in Ctenophores, Cnidarians, and Bilaterians presuppose a nervous system. The evolutionary origin of bioelectrical mechanisms used in neurons seems to be non-neural cells that guide an organism through state spaces (McMillen & Levin, 2024; Prindle et al., 2015) or 3D space (Leys, 2015). In embryogenesis and tissue

regeneration, it helps the different parts of the organism to locally organise and navigate a virtual morphospace. This capability could then have been evolutionarily **co-opted for navigating 3D space motorically** (McMillen & Levin, 2024). Similarly, the morphological development of the body depends on the simultaneous development of the brain (Herrera-Rincon & Levin, 2018), which means that the *neural* systems essentially fulfil the purpose of guiding the organism through morphospace, similar to non-neural bioelectric systems, only centralised. The origin of the nervous system remains uncertain, but it seems that its provision of **long-distance coordination of cell division and differentiation** enables complex morphological development.

1.1.5 Important Methods of Research in Morphogenesis

Wilhelm Roux recognised as early as the end of the 19th century that it was necessary to **intervene** in development **to understand** its mechanisms. Until recently, research on the human embryo was mainly descriptive, focusing on observation and documentation of developmental processes. Now, there are more and more methods being utilised, and bottom-up and top-down analyses are being integrated.

Optogenetics is one of many techniques being increasingly applied in developmental biology. It allows for monitoring and modulation of specific cellular activities by detecting photo-sensitive proteins that control signal transduction. It provides high spatiotemporal resolution as highlighted by Bugaj et al. (2017). By precisely manipulating the genetic and molecular components of cells in real-time, optogenetics provides many different tools to study developmental processes and possibly reconstruct morphogenesis (Krueger et al., 2019).

Generative models, as discussed by Stillman & Mayor (2023), represent another significant advancement. These models use algorithms to simulate the processes of embryonic development, enabling researchers to predict how changes at the genetic or molecular level can influence overall developmental outcomes. By integrating vast amounts of data from various sources, generative models offer predictive power. This approach especially helps to identify potential causes and interventions for developmental disorders.

Budjan et al. (2022) and many others have explored the use of **organoids** and **other *in vitro* methods**. Organoids are three-dimensional conglomerates grown from TDCs or iPSCs that

mimic the architecture and function of real organs. These models provide a more accurate representation of human development than traditional two-dimensional cell cultures. Research by Diaz-Cuadros (Diaz-Cuadros et al., 2020; Diaz-Cuadros & Pourquié, 2021) has shown that organoids can be used to study early embryonic development, allowing scientists to observe the formation and differentiation of tissues in a controlled environment. The key advantage of Organoids is being able to bridge the gap between *in vitro* study conditions and *in vivo* complexities.

The field of neural **genoarchitecture**, as detailed by Puelles & Ferran (2012), is an emerging discipline within neuroanatomy that focuses on the genetic blueprint underlying the structural organisation of the brain. While traditionally associated with neuroanatomy, neural genoarchitecture's principles are gaining relevance for developmental biology. This approach involves mapping the expression of genes within the developing nervous system to understand how genetic information translates into the anatomical structures of the brain. By elucidating the genetic control of brain development, neural genoarchitecture contributes to our understanding of how the central nervous system is formed and how it interacts with other developing systems within the embryo.

Besides the aforementioned methods, there are many more being applied in the investigation of morphogenesis. Soaked-bead assay, spatial-statistical, and more widely used methods like confocal microscopy and other imaging techniques are at the core of research on embryonic development. For further investigations of widely used methods, see Aigouy et al. and Multerer et al. (2017; 2018).

1.2 Embryogenesis in Vertebrates

The embryonic development of vertebrates, from the zygote stage to the formation of somites and a neural tube, involves many well-characterised processes that will be described with the necessary detail for this thesis. The goal is not only to provide a sufficient biological account of these processes for the understanding of the practical work performed in the course of this thesis but also a fundamental literary overview of other adjacent morphogenetic processes for faster implementation in the modelling environment.

As vertebrates provide the most conserved and complex account of neurogenesis, we will focus on this subphylum instead of the phylum of chordates. It is most conserved because,

with the rising complexity of a phenotype, the importance of conserving gene regulatory mechanisms increases (Berthelot et al., 2017). It is also suggested, that more complex phenotypes lead to a lower likelihood of mutations increasing complexity (Hagolani et al., 2021). Some examples provided are from specific species exhibiting certain phenomena that are not directly projectable to the whole subphylum but sufficiently comparable for the given context. All processes described in the following may also vary in timing and sequence from one vertebrate to the other but are similar enough to exhibit the same morphogenetic principles (Figure 3). The overview will be a combination of mostly anatomical (Gilbert SF., 2020; Kressin & Brehm, 2019; Sadler & Langman, 2011) and various histological and genetic accounts of vertebrate development.

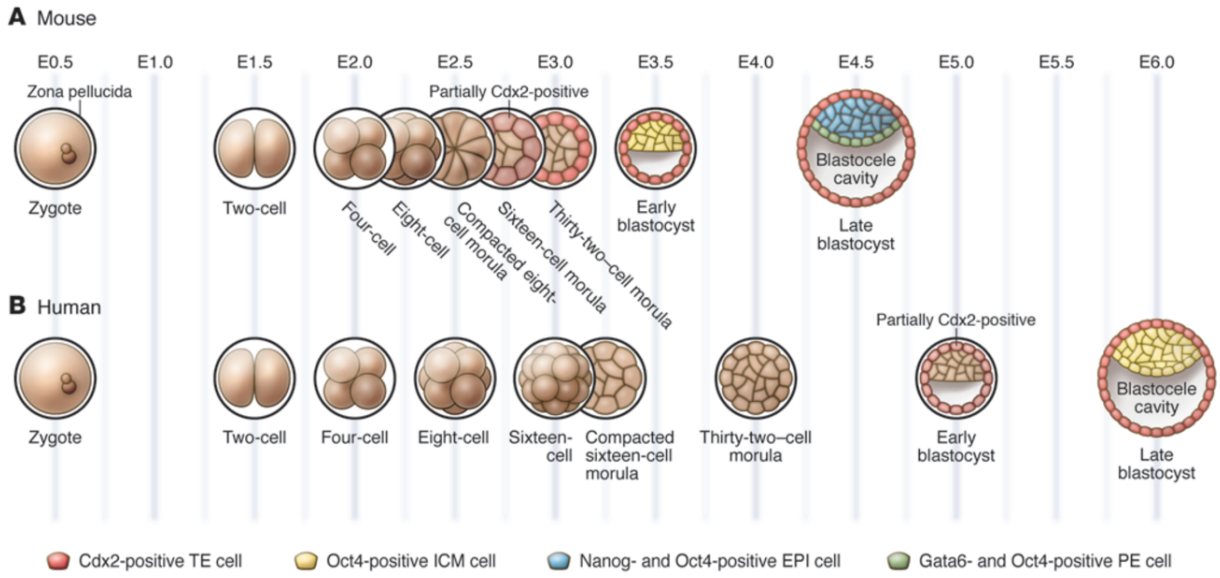

Figure 3: Stages of mouse and human preimplantation development, from (Cockburn & Rossant, 2010).

An overview of the first few typical steps of development in vertebrates. It demonstrates the differences in timing that appear even in phylogenetically more similar vertebrates. The mouse embryo reaches the blastocyst stage already in week three, whereas the human embryo takes five weeks to arrive at the same stage.

1.2.1 Early Embryonic Development

The patterning of the embryo and lineage development and the respective molecular pathways involved are still not well understood. Although the knowledge gaps differ from one animal to the next, no organism is understood well enough, as exemplified by humans (Figure 4). Early embryonic development can be divided into **two main stages**, pre-implantation and post-

implantation separated by the **implantation** of the hatched embryo into the endometrium. This first section will deal with the **preimplantation embryo** within the zona pellucida (ZP).

Once fertilised, the pronuclear egg cell turns into a zygote by fusion of parental nuclei and quickly goes through several iterations of mitosis, a process called **cleavage**. The resulting cells, now called blastomeres, undergo maternal clearance and zygotic genome activation in what is called the maternal-to-zygotic transition at the *two-cell stage* (Lee et al., 2014), whereby the control of the blastomeres through maternal mRNA is transitioned to **self-organisation** by the blastomeres themselves. This process can take anywhere from a few hours to a few days, depending on the animal (Vastenhouw et al., 2019). The initial developmental bifurcation and principle of morphogenetic diffusion in blastomeres is called **first lineage separation** (FLS) and starts at the two to four-cell-stage with first signs of intercellular heterogeneities (Biase et al., 2014; Cui & Mager, 2018), meaning they start to differ in their patterns of gene expression (Ozawa et al., 2012) and thus cell lineage. This process can be approximated by a two-component reaction-diffusion model. Meanwhile, the morula has formed and goes through **compaction** at the *eight-cell stage*, leading to a much denser cell clot. During this stage the physical separation of these lineages happens too, when **Hippo-signalling** becomes active in apolar cells and inactive in polar cells, depending on their actomyosin concentration, which depends on the physical position of the cell in the conglomerate (Cui & Mager, 2018; Lamba & Zernicka-Goetz, 2023). The result of this process is the **polarisation** of cells, consequently leading to a separation of the embryoblast or **Inner Cell Mass** (ICM), which forms the **hypoblast** (future yolk sac) and the **epiblast** (future foetus), from the **Trophectoderm** (TE), which further develops into an integral part of the placenta and other extraembryonic tissues. This process of **blastulation** is completed by more and more intercellular fluid that penetrates the ZP and loosens the ICM in the morula, until a cavity, the blastocoele, is formed. This inevitably leads to the formation of the **embryonic-abembryonic axis** (EAA), where the ICM groups together in the growing blastocoel to attach to the spherical TE structure. Differential properties of adhesion between the ICM and TE cells help to position the ICM at one pole of the blastocyst. Adhesion molecules such as E-cadherin play a crucial role in this process. The causal events leading to the formation of the EAA remain unclear and seem to depend on multiple factors, but there is evidence that the axis of the first cell cleavage already positively correlates with the EAA (Plusa et al., 2005). This marks the end of the preimplantation embryo, as the blastocyst now hatches out of the ZP in the uterus and performs **implantation**, executed by trophoblastic cells above the embryoblast pole burrowing into the uterine epithelium.

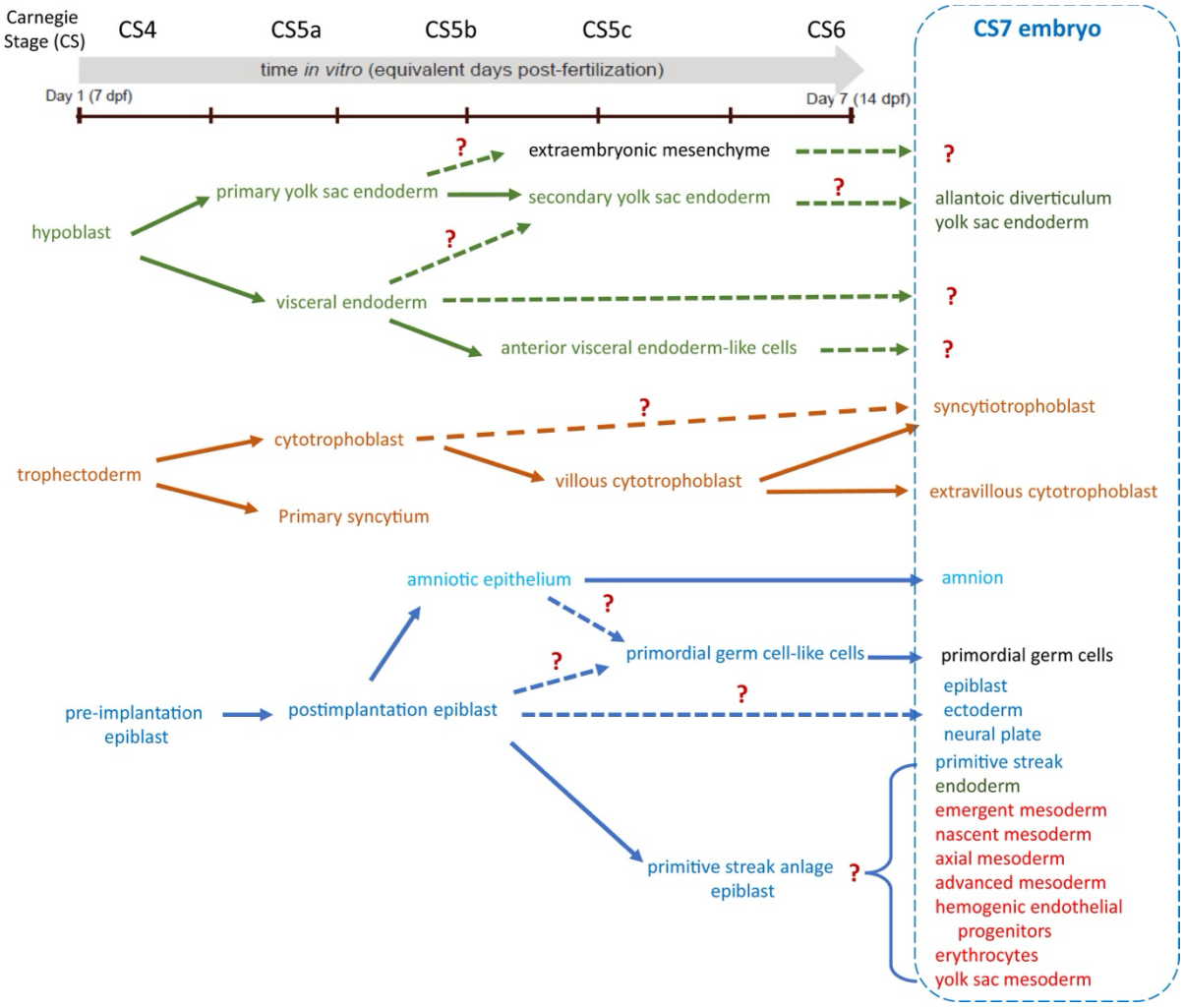

Figure 4: Lineage phylogeny of the human peri-implantation embryo, from (Rossant & Tam, 2022).

The predicted relationships between preimplantation epiblast, hypoblast, and trophectoderm during early implantation are based on anatomical studies and gene activity patterns. The Carnegie stage (CS) and the approximate in vitro timeline for different cell and tissue types are indicated. Uncertain lineage relationships (dashed arrows and "?") between cell types in the peri-implantation embryo and the CS7 gastrula-stage embryo (dashed box) are highlighted.

Gastrulation or Midline Morphogenesis

Introduced by Haeckel in 1866, derived from the Greek word “gaster” (Eng. “gut”), the following process of **gastrulation** leads to the formation of the **three germ layers**. This property of development is also shared among all bilaterians and is also the step, where the bilateral symmetry along the anteroposterior axis gets instantiated. Ultimately, gastrulation is the

process of transformation of the bilaminar disc (hypoblast and epiblast) into **ectoderm**, **mesoderm,** and **endoderm**.

The first step of gastrulation is the formation of cavities both within the epiblast (forming the amniotic cavity, AC) and the hypoblast (forming the secondary yolk sac, SAS) layers, which are joined to the extraembryonic tissues via a connecting stalk. At the adjacent surfaces of those two types of cells, the epiblast cells migrate to a midline to form the **primitive streak** which starts at the most caudal point, where the primitive node forms. This is also called the *Spemann-Mangold Organiser* after Spemann & Mangold (1924) in amphibians, or *shield* in others. It tails off cranially as the **primitive groove** with two parallel adjacent primitive streaks and moves from the caudal cloacal membrane towards the oropharyngeal membrane, defining the **lateral-medial body axis**. The initially almost round shape of the embryonic disc becomes anteroposteriorly elongated and widens cranially. Some of the migrating epiblasts infiltrate the adjacent hypoblast cell layer and will turn into the endoderm, while the remaining ones develop into ectoderm. By a process called **invagination**, most epiblasts travel into the space between the two layers however, forming the mesodermal tissue, of which the **notochord** is the first one. It forms by an intricate process starting with epiblasts moving from the primitive pit to form a **notochordal process** that attaches to the **prechordal plate** cranially. The adjacent **prechordal mesoderm** forms by prior cell taxis of epiblasts also coming from the primitive pit and replacing the hypoblasts of the oropharyngeal membrane and additionally forming a trilaminar structure at the caudal end of the oropharyngeal membrane. The processing notochord forms an inner canal through apoptosis and intercalates with the endoderm as the process reaches the prechordal mesoderm. It opens ventrally, leading to the transformation of the canal into a bow-shaped **notochordal plate** surrounded by intraembryonic mesoderm. This now leads to a temporary connection between the AC and the SAS called the **neurenteric canal** as the primitive pit leading into the former notochordal canal now opened through the endoderm. The notochordal plate then goes through a kind of reverse process, narrowing its vegetal opening before closing it completely and moving up into the centre of the intraembryonic mesoderm again, whilst forming a solid but flexible rod. Now the proper notochord is formed. Its role is to serve as a major signalling source for the further development of structures forming along the anterior-posterior axis (e. g. neural tube and somites) and as a precursor for the skeleton (Stemple, 2005).

Body Axes Formation

An important aspect of early embryonic development is the formation of the **three planes of asymmetric segmentation**: the dorsoventral, anteroposterior, and left-right axis. The establishment of the body axes is a process that takes place before and during gastrulation and is a central phenomenon when talking about morphogenetically guided pattern-forming developmental processes. As discussed in *Maternal Control of Development in Vertebrates* (Marlow, 2010), there are significant differences in the ways different vertebrates establish their body axes, with some utilizing maternal mRNA and others solely relying on zygotic factors. This means that there is no universally valid ontological account for how body axes of vertebrates are formed.

Morphologically, through the formation of the primitive node and on a cellular level through the arrangement of epiblasts and hypoblasts, the **dorsoventral axis** is established at the same time as the anteroposterior axis. Bone morphogenetic protein 4 (BMP4) and the transforming growth factor (TGF-β) family produced across the embryonic disk and mainly its interaction with wingless-related integration site 3 (Wnt3) lead to dorsoventral patterning (Abas et al., 2022).

The **anteroposterior axis** establishes head and tail directions before gastrulation. The anterior primitive streak and the anterior visceral endoderm (AVE) express genes for head and tail directions and secrete effectors like Nodal. These effectors are involved in primitive streak formation and mesodermal tissue differentiation, through pathways such as the TGF-β family, Wnt signalling, retinoic acid (RA), and FGF-BMP interactions. It should be noted that especially Nodal is thought to have originated to specify the oral region and does not affect mesodermal tissue differentiation (Whelan et al., 2017).

The third axis to be established is the **left-right axis**. A recent preprint (Asai et al., 2024) among other sources (Shiratori & Hamada, 2006) points towards cilial movements called “polonaise movements” leading to a leftward flow of extra-embryonic fluid. This mechanism could be the reason for the early left-right asymmetry, prior to the appearance of laterality gene expression of key effectors like *LEFTY*, *FGF,* and serotonin (5HT) around the primitive node, at the so-called Left-Right-Organiser (Hamada & Tam, 2014). Although recent years have led to many important contributions around this topic. As pointed out in the preprint, it is currently still unknown how the described flow is induced, thus not being an ontological account of bilateral symmetry breaking.

1.2.2 Early Development of the Nervous System

1.2.2.1 Neural Induction and Neurulation

Above the notochord in the ectodermal layer, neural induction forms the **neural plate** due to signals from the notochord and adjacent mesenchyme (DeSesso & Williams, 2018). This process distinguishes neural ectoderm from non-neural ectoderm. Shortly after, ectodermal cells at the border transform into the neural crest. The neural plate invaginates, creating a **neural groove** that narrows as ectodermal folds meet, leading to **primary neurulation**, where the neural groove forms the **neural tube** and **neural crest cells**. Neural tube closure is enabled by **neural fold zippering** (Figure 5), proceeding from posterior to anterior, with long filopodial processes forming temporary bridges that pull together to close the tube tightly. The key morphogen coordinating this process is Integrin β1 (Molè et al., 2020).

The process concludes with the closure of the anterior and posterior neuropores. The neural tube cells form the central nervous system (CNS) while the neural crest cells form the peripheral nervous system (PNS). Neural tube closure starts at the fourth or fifth somite pair, marking the future border of the brain and spinal cord, and proceeds both caudally and cranially. Since the neural tube initially extends only from the oropharyngeal membrane to the primitive node, **secondary neurulation** extends it caudally to the cloacal membrane, a process unique to vertebrates and linked to tail elongation. The caudal part of the embryonic disc, called the tail eminence, reorganises mesenchymal cells to form a solid cell cohort. Secondary neurulation involves cavitation to form the medullary tube, which attaches to the primary neural tube, a process seen in amniotes like humans (Saitsu et al., 2004) and conserved in non-amniotes like amphibians (Gont et al., 1993).

The morphogenetic basis of neurulation involves the interactions of a few key regulatory mechanisms (Figure 6), all based on BMP-signalling. The two resulting cell shape alterations are **apical constriction** and **basal nuclei location**. Paired with higher cell proliferation in hinge regions (McShane et al. 2015), these mechanisms afford the formation of different hinge point cells to create the necessary bend.

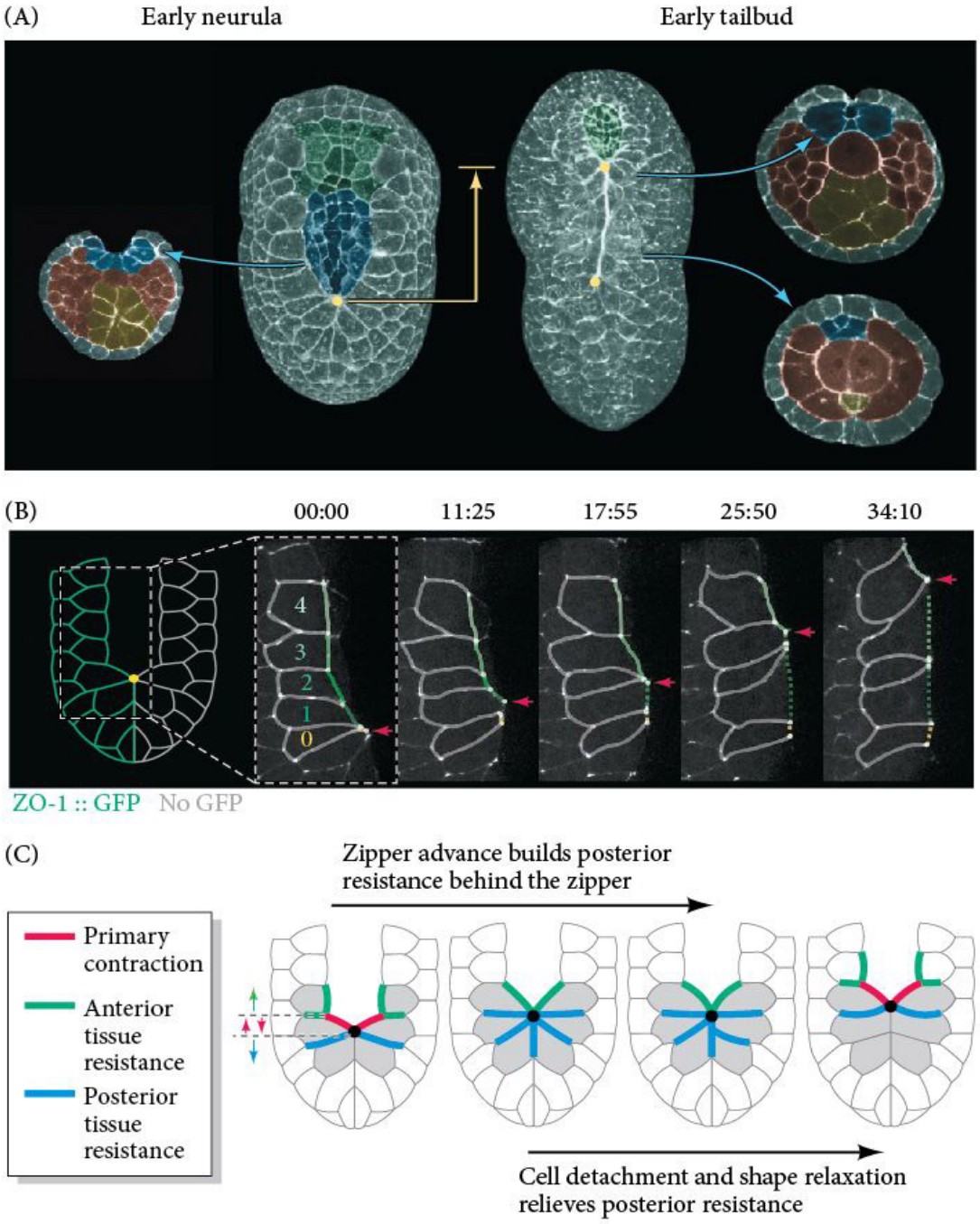

Figure 5: Neural tube zipper advance in Ciona, from (Hashimoto et al., 2015).

Morphogenesis guides an intercellular mechanical process. Modelling this in Morphogenesis would prove the program can model intricate mechanics.

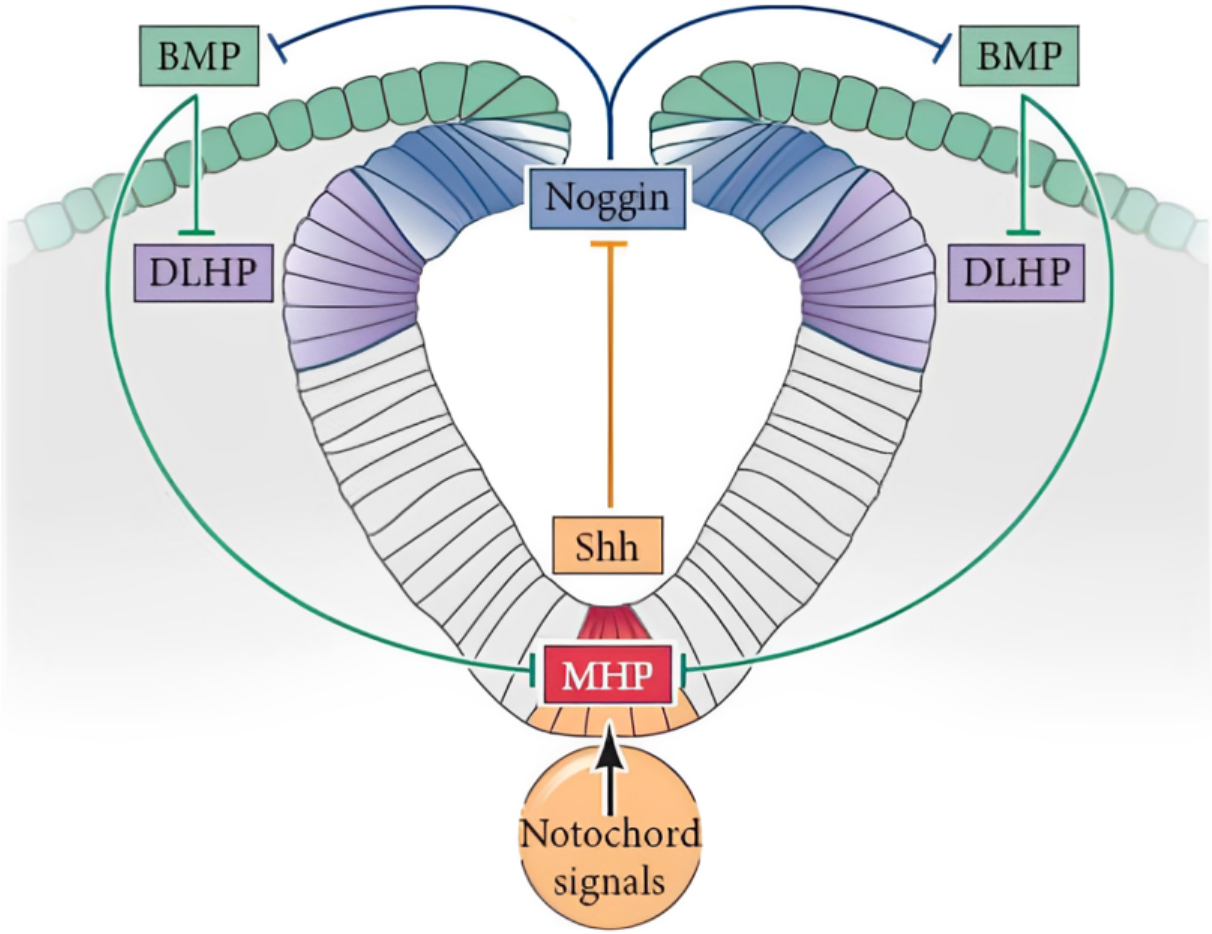

Figure 6: Morphogen regulation of hinge point formation, from (Gilbert SF., 2020).

BMPs are expressed by the surface ectoderm (green), Noggin is expressed in the dorsal neural folds (blue), and Shh is expressed ventrally in the notochord and floor plate (orange). The regulation of hinge points revolves around BMP as an antagonist to both DLHP and MHP formation. Shh is required for the specification of the floor plate, while additional signals from the notochord induce MHP morphology. Noggin directly inhibits BMP ligands, thus alleviating BMP repression of the hinge points. The DLHPs, however, form only at the correct size and dorsal-ventral position, which is based on Noggin's distance from inhibitory Shh gradients ascending from the floor plate. Therefore, apical constriction occurs only in those cells experiencing low enough concentrations of both BMP (MHP and DLHP) and Shh (DLHP) morphogens. From (Gilbert SF., 2020) quality is enhanced with fotor.com.

1.2.2.2 Neural Crest Development

In the following developmental step, neural crest cells form a thickening layer between the ectoderm and tube and start migrating laterally to form various tissue types, including adipocytes, smooth muscle cells, chondrocytes, osteocytes, odontoblasts, melanocytes, sympathoadrenal cells, Schwann cells and peripheral neurons of course (Rao & Jacobson,

2006). The significance of the neural crest cannot be overstated, which is why some authors call the neural crest the **fourth germ layer** (Barresi & Gilbert, 2000). The signalling pathways involved in this process are mainly *FGF*, *Wnt,* and *BMP* and their interaction (Leung et al., 2016). The neural crest specifiers are *Slug* and *Twist*.

Dynamical Systems Theory will be introduced in a bit more detail in the context of the next key step in the development of the embryonic nervous system, somitogenesis. This allows for a better linkage with the developmental morphogenetic processes and additionally provides a firmer scaffold for the practical part of this thesis. This chapter will include a general overview of somitogenesis and the **segmentation clock**, as review papers on this topic have already been published (François & Mochulska, 2024; Gomez et al., 2008; Miao & Pourquié, 2024). Although comparable processes occur in all chordates, annelids, or arthropods, segment formation has key differences such as tissue of origin, gene expression patterns, and evolution. For more detailed distinctions between subphyla, see Clark (2021). The following treatment applies to the vertebrate subphylum.

1.2.2.3 Dynamical Systems Theory

Three principles of pattern formation that can be applied to somitogenesis and various other morphogenetic processes lie at the core of Dynamical Systems Theory for modelling morphogenesis.

1. Positional Information PI

Originally proposed by Wolpert (1969), and as a modern concept through the lens of information theory by Tkačik & Gregor (2021), strong PI at its core describes a cell parameter that represents and directly correlates with a coordinate position of the cell and determines cell differentiation. This also determines the cell’s fate. Wolpert illustrated this concept with the French Flag problem, which was only extrapolated to a model later. It describes a morphogen gradient across a tissue determining gene expression patterns analogous to the French flag's three colours (Figure 8, B). Wolpert himself initially only used it to define a problem (Sharpe, 2019). He also proposes an alternative to the French Flag approach called the balancing model that does not utilise gradients nor positional information, but solely relies on self-organisation and reaching the same goal of size-regulated Turing-pattern formation. Cells interpret their

position within this gradient, differentiating into different cell types based on threshold concentrations of the morphogen, much like the distinct colour bands on a flag.

2. Self-Organisation

Self-organisation in biological systems refers to reaction-diffusion (RD) processes, a concept theoretically hypothesised by Alan Turing (1952) and experimentally proven by Castets (1990) and Ouyang & Swinney (1991), among others. Self-organisation occurs through different combinations of positive and negative feedback loops, resulting in diffusion gradients influenced by stochastic differences in cell parameters. One notable model of pattern formation through reaction-diffusion is the activation-inhibition mechanism proposed by Gierer & Meinhardt (1972). This model involves short-range enhancement and long-range lateral inhibition enacted by each cell, leading to complex patterns within the biological system. Various mechanisms can lead to pattern formation through self-organisation, as summarised by Landge (2020). The integration of these concepts has been further discussed by Green & Sharpe (2015), highlighting the fusion of different self-organisational mechanisms.

3. Differentiation Waves

The concept of Differentiation Waves, first introduced by Cooke & Zeeman (1976) as the clock-and-wavefront mechanism, provides spatial and temporal information to different parts of tissue through established organisers, origins of morphogen diffusion. Essentially, differentiation waves act as a stimulus that propagates through the tissue, providing cues critical for appropriate cell fate determination. This will be explained in more detail during the discussion of somitogenesis.

Utilisation of these Principles

In developmental molecular patterning in general, there are real examples of patterning systems that can be best described by solely one of these models and others that can be described by a specific combination. There is no definitive answer as to what model explicitly or implicitly represents the reality of somitogenesis most adequately. Some authors argue for a combination of RD and PI to be suitable for modelling any pattern formation mechanism (Green & Sharpe, 2015; Reinitz et al., 2023).

Clark (2021) provides a good overview of the core principles of segment formation. He summarises the recent empirical findings, as well as doing a more detailed analysis of

underlying morphogenetic processes and presenting coarse-grained models to simulate the dynamical processes of the constituents of the segmentation clock. He points out that, although most current models implicitly assume a PI framework, where cells continuously adapt their internal state to their surrounding morphogenetic stimuli, empiric work points to a more discretised state space with unstable intermediates and a partially decoupled relationship between environment and internal state of cells. Despite all this, he goes on to show that modelling a steady-state scenario with identical spatial and temporal coordinate systems suffices to approximate somitogenesis, as both time- and space-based approaches often lead to comparable outcomes.

1.2.2.4 Somitogenesis

During the development of the neural tube of all vertebrates, the intraembryonic mesoderm differentiates into three distinguishable layers laterally to the central axis which consists of the notochord and the neural tube, namely into **presomitic (paraxial)**, **intermedial** and **lateral mesoderm** from medial to distal on both sides of the notochord. In **segmentation**, presomitic mesoderm (PSM) is segmented into isolated bodies called somites along the anteroposterior axis, although the most cranial part remains PSM, only partially segmenting into somitomeres. The embryo concurrently grows along the anteroposterior axis due to continuous cell proliferation and ingression in the tail-bud region. Each somite can itself be divided into dorsal dermomyotome and ventral sclerotome. The latter forms the vertebrae, which protect the spinal cord, and the ribs, who are protecting the heart, lungs, and major vessels.

Of all proposed models of somitogenesis, the **clock-and-wavefront model** (Cooke & Zeeman, 1976) proved to be the most accurate. Consisting of a molecular oscillator, the clock, and a diffusion gradient, the wavefront, it coordinates the periodicity of somitogenesis along the paraxial mesoderm by transcription factor gene expression. It works by producing a pattern of morphogen diffusion along the anteroposterior axis. Originating posteriorly, the effectors of the clock (*Notch effectors like hairy*, *hey*, *hes or her*) provide the temporal component, whereas the wavefront (*Wnt*, *FGF8 and others, depending on the species)* provides the spatial component. As soon as the segmentation clock is in phase and reaches the cells that are below the critical threshold, the **determination front**, they start to segment. The sole challenge presented by this model lies in elucidating the termination of segment formation. This is because the model depends on a constantly elongating tail of PSM, which allows the wavefront to move with it and pull back the determination front (Figure 7). If somitogenesis ends, it must

be terminated such that all the PSM differentiates. This is addressed by Pantoja-Hernández et al. (2021) through the hybridisation of the clock-and-wavefront model and a progressive oscillatory reaction-diffusion (PORD) model based on the work of Hand Meinhardt. This integration of models would lead to a very accurate model of somitogenesis when implemented into Morphogenesis. Although somitogenesis is highly conserved in all vertebrates and other subphyla of chordates, the number of segments varies drastically between species and does not correlate with the total duration of somitogenesis (Gomez et al., 2008).

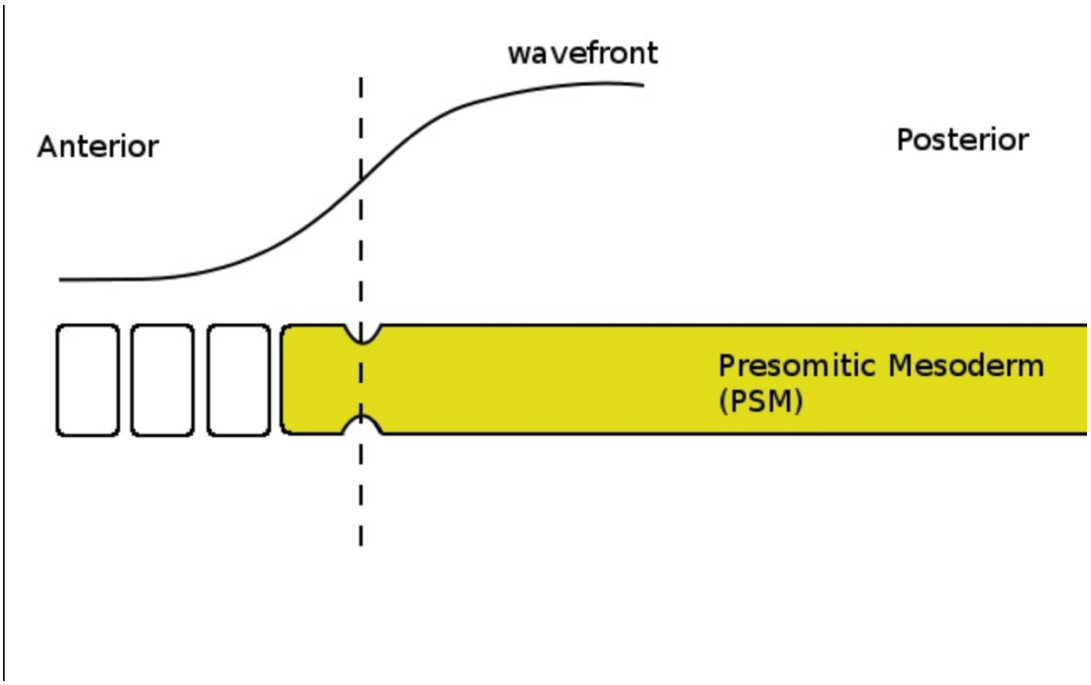

Figure 7: Schematic of somitogenesis.

The determination front, where PSM cells transition into somite cells, is established and depends on the wavefront moving posteriorly, reducing the concentration of the morphogen to a level that allows the PSM cells to differentiate.

During segmentation, a crucial process in identifying somites is **regionalisation**, the activity of a group of homeobox genes called **Hox genes**. All species examined thus far exhibit Homeobox gene regulation to be conserved across major metazoan groups. Hox genes are expressed in patterns along the anteroposterior and the dorsoventral axis. They serve to separate groups of axial segments (Mallo et al., 2010), such as cervical, thoracic, lumbar, sacral and caudal. In both vertebrates and arthropods, Hox genes are expressed sequentially from 3' to 5' along a gene cluster and specify segment identities (Deschamps & van Nes, 2005; Iimura & Pourquié, 2006; Izpisúa-Belmonte et al., 1991). Expression starts before

somitogenesis at the primitive streak and stops about mid-gestation. This segment identification along the body axis works by decondensation of increasing amounts of Hox genes expressed in the PSM with respect to time. The intergenic sequences in between determine the time that passes from the activation of a Hox gene to the next (Montavon & Duboule, 2013), leading to a more uncoiled DNA the more posterior a cell is positioned (Chambeyron & Bickmore, 2004).

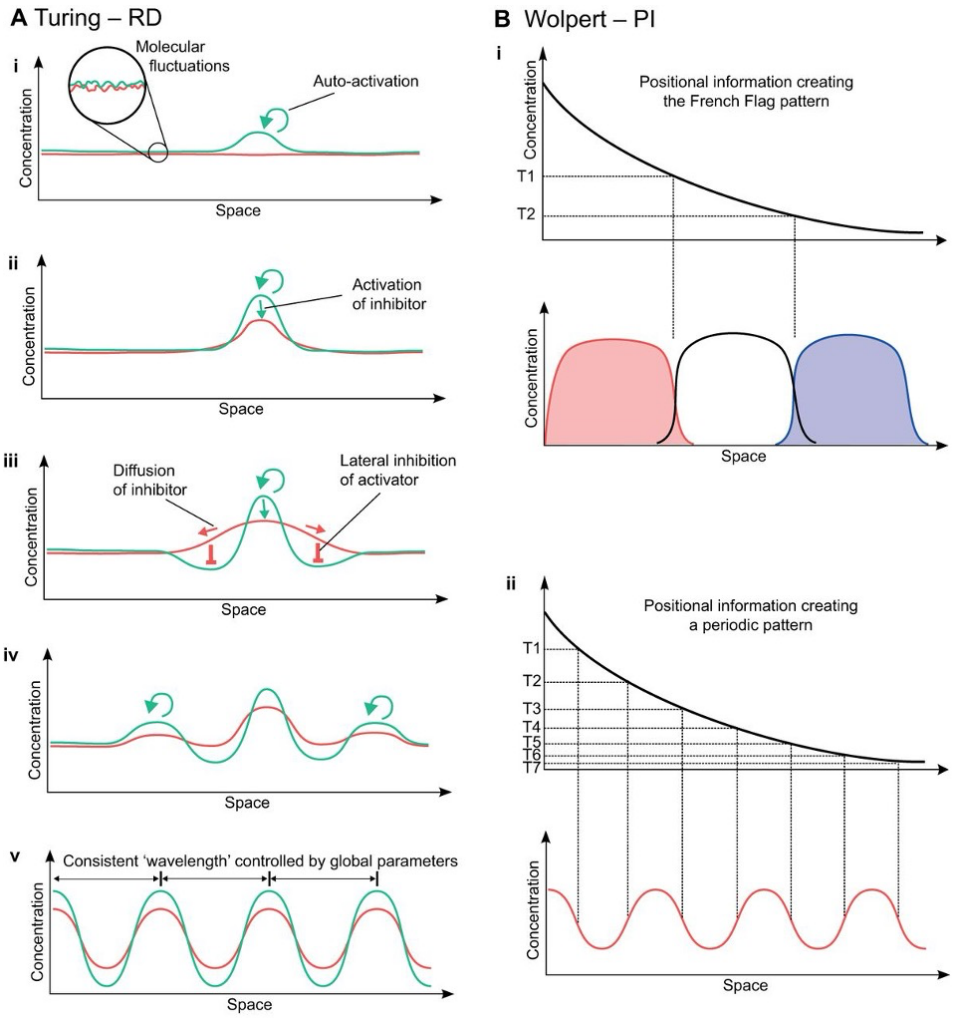

Figure 8: The principles of RD and PI systems, from (Green & Sharpe, 2015).

The key difference to note between these two processes is that RD is a global pattern, forming from only stochastic differences, without an organiser, while PI relies on a local source or secretion.

1.3 Fluid Simulation

We have already established biology or life itself to be a complex interplay of various far-from-equilibrium steady-state systems. A model of a system always compromises accuracy for simplification. Many theoretical, mathematical models and physical simulations of the morphogenetic process have been reviewed (Grodstein et al., 2023; Stillman & Mayor, 2023; Y. Wang et al., 2020; Wyczalkowski et al., 2012). In general, there are a lot of studies examining various models and their respective processes in isolation or providing mathematical foundations. Many focus on a purely mechanistic level of modelling. The Morphogenesis simulator being established by Nick Hockings at the PLF-Hub at the Institute of Animal Welfare Science of the University of Veterinary Medicine Vienna takes a novel approach by integrating the modelling of form and many different principles of heredity. It is computationally based on a large-scale, open-source fluid simulator (Wu et al., 2018) called "**FluidsV3**" (Rama Hoetzlein, 2012), which applies the **smoothed-particle hydrodynamics** (SPH) method (Desbrun & Gascuel, 1996) integrating Lagrangian mechanics and Eulerian mechanics (Foster & Metaxas, 1996), first combined by Stam (1999), put into context in Figure 9. The approach of "hydrodynamics" in general has led to a lot of advances in many fields that study far-from-equilibrium systems like weather phenomena, traffic flow, or ecosystems (Jaeger & Liu, 2010). The foundation of SPH programs, or hydrodynamics in general, are the **Navier-Stokes equations**, describing the behaviour of fluids through the conservation of momentum, mass, and energy. Morphogenesis' central quality can be interpreted as coupling microscopic and macroscopic scales, as described by Krinsky (1984). FluidsV3 is capable of modelling up to eight million particles on the GPU by applying the fluid-implicit particle (=FLIP) method (Brackbill & Ruppel, 1986), an improvement on the fundamental particle-in-cell method by Harlow (1962).

1.3.1 The Morphogenesis Simulator

The Morphogenesis program has been developed to simulate "soft-matter elasticity, diffusion of heat/chemicals/morphogens, epi-genetics and particle automata behaviour" (GitHub-code by Hockings & Young, 2024). Through genomic variation it is expected to produce smooth changes in phenotype within certain ranges, thus supporting an evolvable genome. This expectation is grounded in established mainstream theories in morphogenesis and based on

Dynamical Systems Theory. In broad mathematical terms, a dynamical system refers to a system in which a fixed rule describes the time dependence of points in a geometrical space.

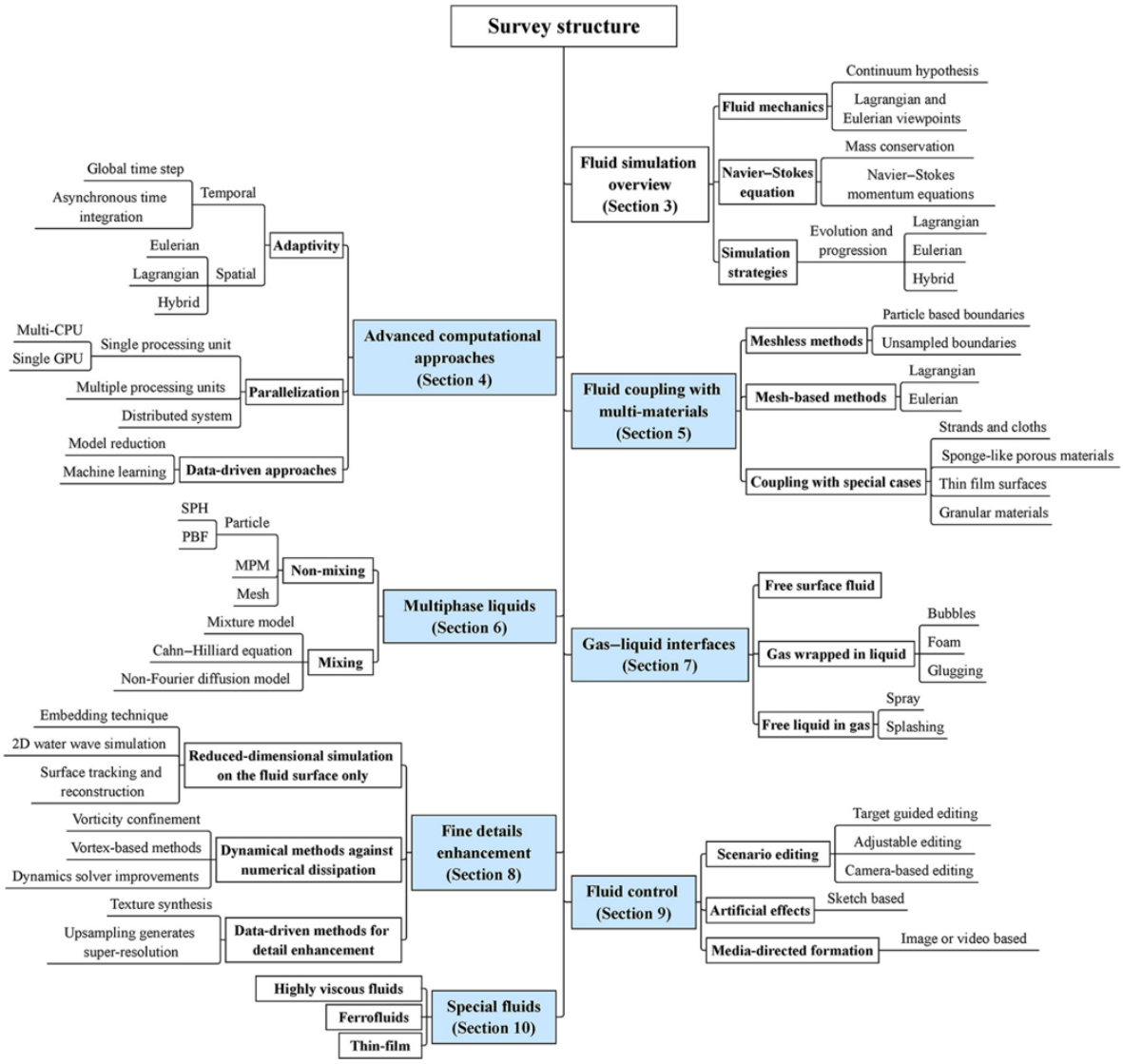

Figure 9: Physics-based Fluid Simulations, from (Wang et al., 2024).

This demonstrates the intricate environment of physics-based fluid simulations. The first branch depicts the modelling principles at the core of Morphogenesis. There are a lot of different methods being applied in physics-based fluid simulations, each with its own areas of application but often based on similar mathematics.

To effectively set up a simulation for each morphogenetic mechanism to be studied, one should begin with simple examples and conduct experiments to determine the range of parameters that produce stable behaviour and the system's response within that range. This can be

achieved by performing parameter sweeps through batches of simulations. Parameter sweeps make it possible to find the right values for parameters to run a successful simulation with. This work was planned to be the practical part of this thesis. I was part of the PLF-Hub Research Group at the Institute of Animal Welfare Science headed by Prof. Jean-Loup Rault for one year, assisting with the translation of Morphogenesis from the CUDA to the OpenCL programming language for Hockings to integrate it with other OpenCL programs.

The Morphogenesis simulator offers significant advantages for studying complex biological processes by providing a controlled environment. Variables can be systematically manipulated and through its computational power, the problem space of parameters can be explored much more efficiently. The primary input data for such simulations are (epi)genomic data, gene regulatory mechanisms, particle properties, and environmental parameters. This allows researchers to model morphogenetic processes at the intersection of genomic data and resulting intercellular mechanisms. The benefits of this approach include the ability to explore how small changes in input parameters affect the behaviour of the system as a whole, which would be less tractable to achieve in physical experiments and unrealistic to model in computationally less efficient systems due to the complexity and scale.

There are several key points behind this modelling approach (Hockings & Howard, 2020). First, DNA patterns affecting mutability can lead to the canalisation of an evolvable body plan. Canalisation refers to the reduction of phenotypic variation in a population, even when individuals possess genetic differences. Second, Hockings and Howard explored various morphogenetic mechanisms, such as epigenetic cell lines, which provide functional cell types and facilitate the identification of cell descent. Epigenetic modifications are heritable changes that alter gene expression but do not alter the DNA sequence. Another mechanism involves the establishment of local anatomical coordinates based on the diffusion of morphogens, which enable the evolvable genetic parameterisation of complex phenotypes. Finally, remodelling in response to mechanical forces supports the robust production of well-integrated phenotypes that are more complex than the genome alone could specify.

The simulator setup requires the definition of three primary files: the **genome file**, specifying the genomic information; the **particles file**, containing information on particle's bonds, epigenetic state, and concentrations of morphogens and transcription factors; and the **simulation volume file**, outlining the modelling space, specifying which components of the

simulator are active, and indicating where to save the output files. The output from the simulator can be visualised using **ParaView**, and the data can be analysed in a spreadsheet for sufficiently small simulations. Once individual mechanisms can be reliably reproduced, the next step involves combining these mechanisms to build more complex systems. By employing the Morphogenesis simulator and understanding these key points, researchers can gain deeper insights into the dynamic processes that underpin embryonic development and the evolution of complex organisms.

2 Aims

The initial aim of this thesis was to summarise morphogenesis, developmental biology, and conceptual frameworks relevant to implementing the first morphogenetic processes in Morphogenesis. This in turn would prove the Morphogenesis program to be a useful tool in researching simulation of morphogenesis and allow subsequent research to build upon my work.

Due to unforeseen circumstances, it was not possible to have a working version of Morphogenesis ready in time for it to be utilised for this thesis. The process of translation took far more time than anticipated, forcing us to fall back on the CUDA version for simulations. As this was not planned, there was not enough time to prepare the CUDA version for my exact purposes. What remains as presentable work are the Python programs written as technical outlines for the real implementation in Morphogenesis. These pieces of code try to **mimic the underlying mathematical principles of diffusion** in a more compact and easier-to-navigate piece of code **to establish the right parameters** for the Morphogenesis implementation. As this was not yet verifiable, we have yet to see how transferable the Python parameters are. An important difference between the Python scripts and possible Morphogenesis-implementations is the lack of possibility of performing parameter sweeps. This describes the process of iteratively running the same simulation with a range of values for different parameters, to find the ones leading to the desired outcome. In the Python scripts, points are **static,** and parameters were explored **manually**, which works well enough for the level of complexity that was implemented but would not be the desirable approach with simulations that build upon more complex interactions of variables, i. e. MGs, TFs, and epigenetics.

As the utilisation of the simulation software was technically not feasible, it was necessary to aim for a pre-modelling of morphogenetic processes and to provide a more theoretical groundwork for further practical research. A lot of fundamental information and sources are provided for an early period of development in vertebrates and the current state of research and corresponding methods and tools are summarised. Additionally, underlying concepts and frameworks are reviewed and used to contextualise the idea behind the practical work. Overall, the aim was to build a foundational work that demonstrates the feasibility of the idea behind the Morphogenesis simulation. After conceptual work and practical scaffolds built by others, this thesis serves as a basis for easing practical implementations in the program. It tries to

answer the first questions that arise when beginning to work on the subject and categorises and identifies critical information for a fundamental understanding.

Apart from gathering theoretical literacy central to morphogenesis in general, two questions are being addressed hereafter. What morphogenetic mechanisms work best for the simulation of first lineage separation, pole selection, and somitogenesis? What steps should be taken to move towards the implementation of morphogenetic processes in the morphogenesis program?

3 Technical Setup

For the work on the Morphogenesis fluid simulation, a local workstation running on the open-source Linux-based operating system *Ubuntu* 22.04 LTS was used. The code is all written in C/C++ and CUDA because it is designed to leverage the high performance of Nvidia GPUs. The IDE KDevelop was used as a programming environment, and CMake with Unix Make was used as the build system. CMake allows it to be built on any OS with any C++ compiler, and a variety of alternatives to Unix Make. The GCC compiler was used for the host code, i. e. code that runs on the CPU, and the Nvidia NVCC compiler for the kernel code, running on the GPU. The workstation runs on an Nvidia GeForce RTX 3080 GPU and has an Intel core i9-10900X CPU (at 3.70GHz x 20 cores). It is also running CUDA on the Nvidia GPU driver. Files are written and read using the VTK and Boost libraries, which ensures that the program is operating system agnostic.

On a single GPU, the simulation size is limited by the RAM onboard the GPU, the device's global memory. The amount of memory that is required per particle depends on the number of morphogens and the number of bonds per particle. Morphogenesis would use about 200 bytes of GPU global memory per particle for a "rich" simulation with 32 genes, 32 morphogens or transcription factors, and fibro-elastic mechanics. A typical GPU such as the RTX 3080 can run over a million particles. For any given GPU, the maximum number of particles in a simulation can be found by dividing the device's global RAM by the data per particle. The processing time per simulation time step depends largely on the number of particles and the number of morphogens diffusing between particles. Diffusion is approximately as expensive as hydrostatic pressure to compute, requiring exchanges between all particles in range.

For the Python scripts, a MacBook Air 2020 M1 was used. All simulations were conducted on macOS Ventura 13.2.1 (22D68), utilizing the IDE *PyCharm Edu* by *JetBrains*. The method applied was to create a mathematically similar enough environment for calculations of morphogen diffusion and gene interactions, while ignoring the mechanical methods established in Morphogenesis. Thus, all simulations were performed with immobile "cells" that iteratively calculate their morphogenic effects on surrounding cells and themselves. It was also designed such that visualisations of a 3D space were possible. The SPH aspect of spatially restricting this effect is effectively implemented as well through an exponential boundary condition on diffusion.

4 Results

In the following, the visualisations generated by the Python scripts are presented alongside a high-level explanation of the simulated process. The code is not explained in detail, as this would be outside the scope of this thesis. For further information about the inner workings of the code, see the supplementary material which includes all the Python scripts used filled with explanatory comments to follow the program flow more easily.

4.1 First Lineage Separation

The results of the first Python script are depicted in Figure 10, each point represents a cell. They were placed randomly within a sphere to represent an undifferentiated early blastocyst. The goal of this script is to simulate the differentiation into TE and ICM. Each cell has three different parameters. The first is a stochastic factor representing all normally distributed probabilistic differences in internal cell physiology, like surface receptor concentration or protein expression rate. The second one is the sensitivity of each cell to the extracellular morphogen. The last one is the concentration of the morphogen within the cell. As every cell has about the same rate of morphogen secretion as determined by the first parameter, this mechanism leads to the highest intracellular concentration of the morphogen in the very centre of the conglomerate, as seen in Figure 10 (C). The further outward a cell is placed, the lower the concentration. The resulting cell fate determination is defined by a threshold in the concentration. As only the very outer layer will differentiate into TE, the difference in concentration between prospective TE and ICM cells is practically discrete.

4.2 Pole Formation

The second Python script models the formation of the pole of the blastocyst. The pole is where the ICM will attach to when the blastocyst forms the blastocoel and where the epiblast will create the amniotic cavity. This is modelled by the implementation of a two-component rection-diffusion model after Turing. This means that there is a transcription factor (TF) and a morphogen (MG) being secreted by each cell. The TF acts autocrine, whereas the MG acts paracrine, diffusing to neighbouring cells. The further away the morphogen secretion happens, the weaker the effect [Figure 8 (a)].

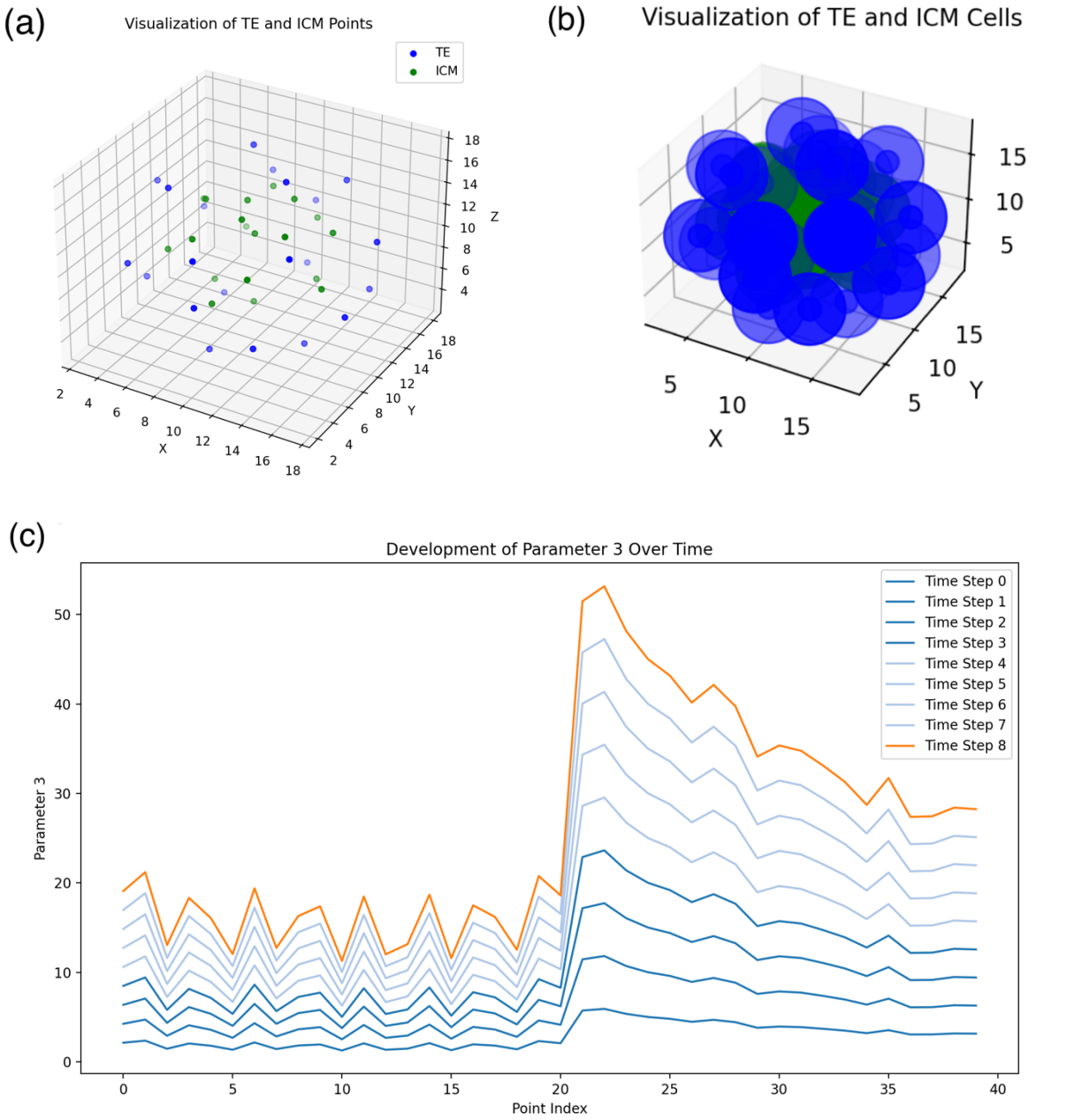

Figure 10: Parameter outputs and visualisations from the first Python script "FirstLineageSeparation.py".

(a) Point Cloud of the position of cells of a blastocyst. They are coloured in with respect to their corresponding lineage. The more translucent a point is, the more distant it is from the viewer, indicating the spherical shape of points that were generated initially. (b) Another form of representation: Cells with nuclei. This better demonstrates the spatial organisation of TE and ICM cells within the conglomerate. (c) A graph that represents the development of the morphogen concentration (=Parameter 3) over time (indicated by the colour of the line) for each cell represented by the point indices. Different functions in the Python script were utilised to provide the visualisations of (a) and (b).

Depending on the parameters set, the number of peaks varies. For pole selection, the parameters need to be set so that across the surface of the cell conglomerate, only one peak appears (Figure 11). This is specified as the “wavelength” in Figure 8 (a). As explained, it was not possible to implement parameter sweeps, and values for diffusion-, MG-, and TF-coefficients were tested manually. To increase consistency and accuracy, a parameter sweep should be performed.

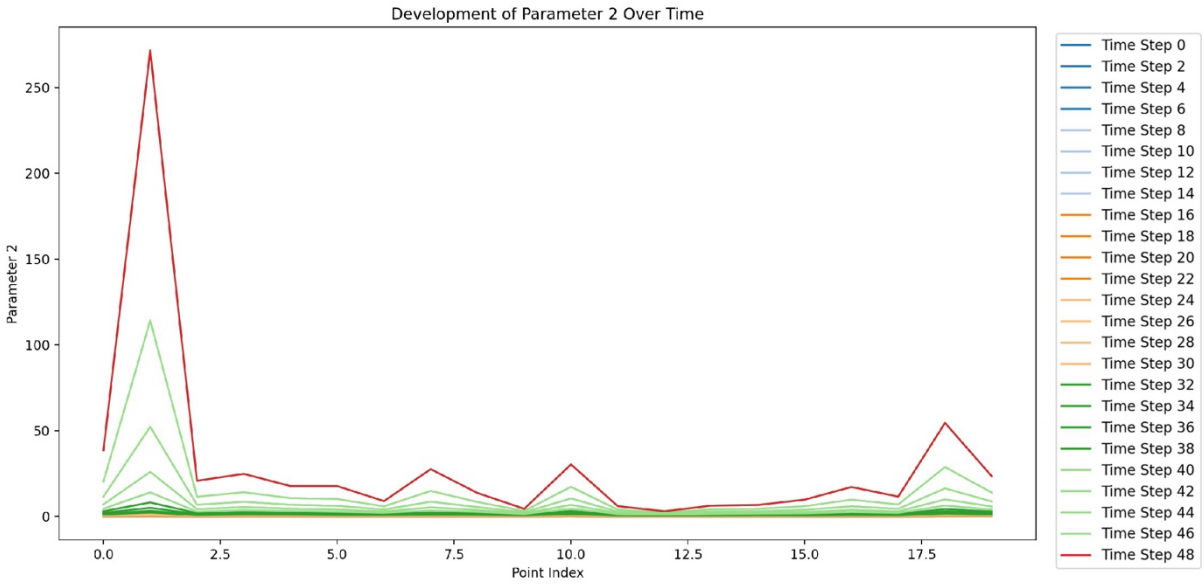

Figure 11: Parameter outputs and visualisations from the second Python script “PoleSelection.py”.

The development of the transcription factor concentration (= parameter 2) over time (indicated by the colour of the line) for each cell represented by the point indices.

The results show a well-working implementation of a two-component RD system that facilitates a single peak on the morula surface from stochastic differences.

4.3 Somitogenesis

The third script tackles the process of somite formation along the neural tube. A few simplifications are necessary, including fixing cells to a position in space. Restricting cells to be static does not affect the process of pole selection or FLS too much, but it is a central part of real somitogenesis. The PSM continually elongates through proliferation along the anteroposterior axis during somitogenesis. As explained in the introduction, the necessary restriction of the length of the PSM does also not allow for proper simulation of the termination of somitogenesis, which is why a simple clock-and-wavefront mechanism was modelled, upon which integration with the PORD model can be established.

The program begins by spawning a definable number of cells in the shape of an elongated horseshoe (Figure 12). Every cell has eight different parameters, explained in the code itself at line 484. The uppermost point in the curvature of the horseshoe shape is defined as a pole marked yellow in Figure 12(a), set through parameter three. The secretion of CLOCK in the code, and the standing WAVE [Figure 12 (d)] both originate at the pole. To simulate this organizing centre moving away in anatomical space, the secretion amount of β-Catenin by the pole is linearly reduced with every iteration. The code runs iteratively over every cell and its influence on itself and the surrounding neighbours. Every iteration it checks for two important conditions to be met such that a cell turns into a somite cell. The conditions are (i) a CLOCK concentration above a certain threshold (Figure 13) and (ii) a low enough WAVE concentration [Figure 12 (e) and “wavefront” in Figure 7]. If these conditions are met, the cell will turn into a somite by setting its second parameter to one. A caveat is that the somites themselves don’t get separated anatomically by intersegmental mesenchyme. The time it takes for a new pair of somites to form depends on the chosen values for the parameters. These parameters are listed and described in the Python script and include CLOCK- and WAVE-coefficients and diffusion factors.

During the development of the script, it became clear that this simulation lacks central functionalities of the real process of somite formation. Especially fixing points, or cells, in 3D space makes it impossible to accurately represent the formation of somites, which partly occurs because the origin of secretion, the organiser, moves further away with respect to time. This can be modelled by changing the functionality of secretion. This adaptation should not be

transferred to Morphogenesis, though, which makes the script less usable as a stepping stone for more accurate simulations.

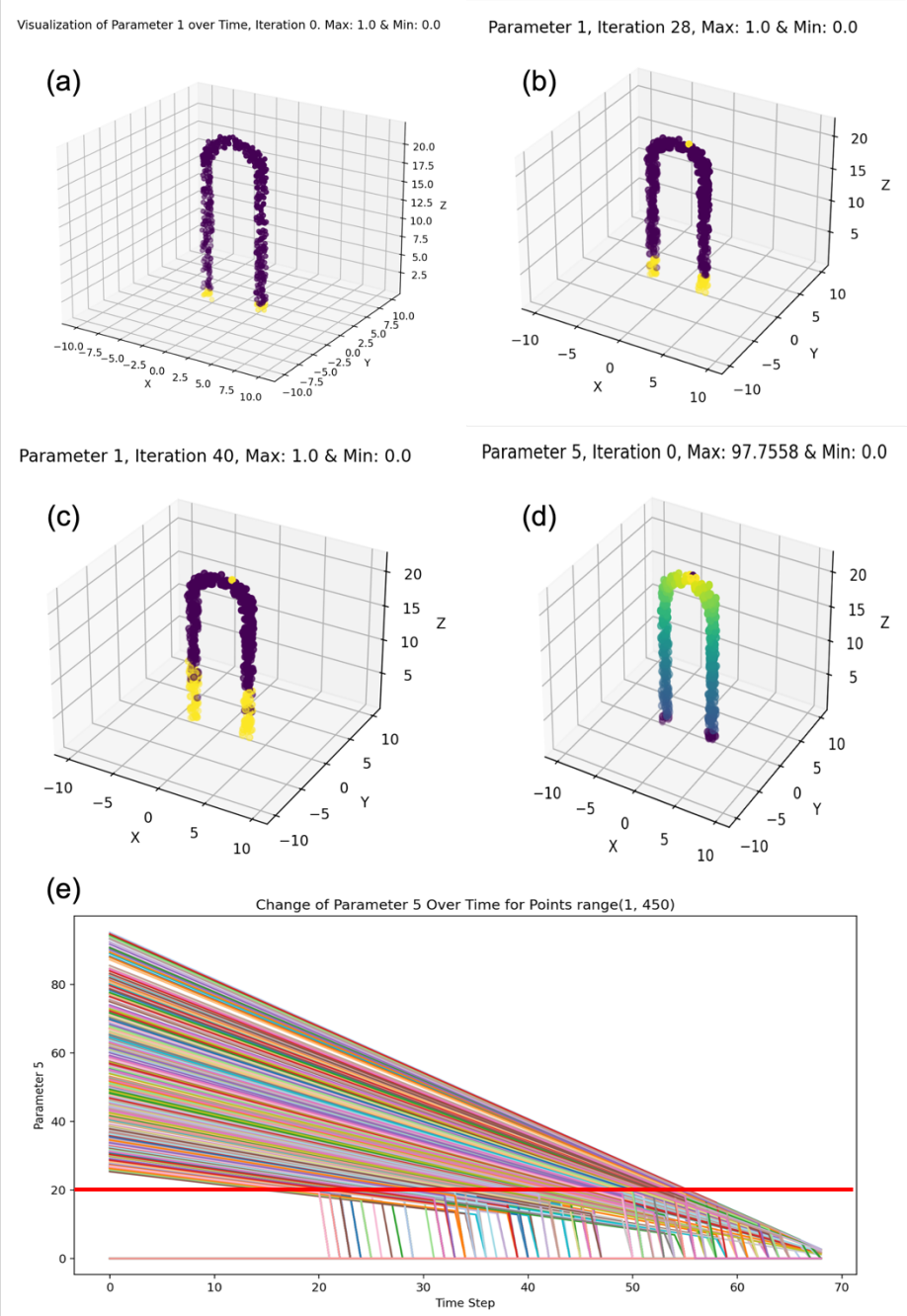

Figure 12: Parameter outputs and visualisations from the third Python script "SomiteFormation.py".

(a) shows the initial state of the PSM, with the first somites activated (= parameter 1) at the bottom (yellow). (b) depicts the progression of somitogenesis, with a new pair of somites having formed at time step 28, and (c) shows the same for the third pair of somites differentiating at time step 40. As parameter 1 is practically a bool, for (a) to (b), the minimum and maximum are one and zero respectively. (d) shows the wavefront originating from the top by colour mapping the concentration secreted from the pole up until the first pair of somites. (e) shows the

progression of the concentration of the wavefront molecule for each cell in the range of one to 450. The threshold value for somitogenesis is set to 20, as indicated by the horizontal red line. Each drop to zero in value for parameter 5 represents a cell turning into a somite.

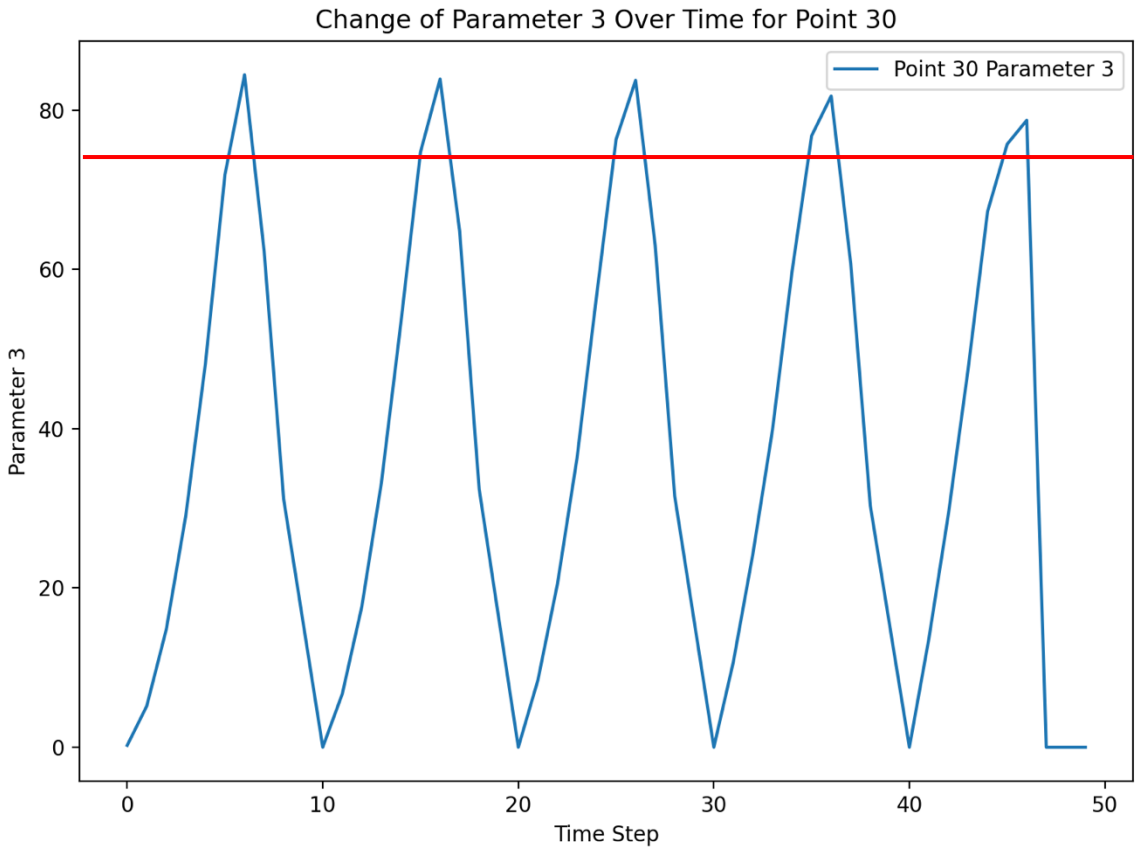

Figure 13: Oscillation of CLOCK (= parameter 3) of a cell (= point) chosen at random.

The steep dip and flat-lining of the parameter at the end indicate the cell's transformation into a somite cell. The chosen threshold was 75 for this simulation, indicated by the red line.

5 Discussion

As already addressed in Section 4 the original objective of this thesis needed to be adjusted while writing. The plan was to utilise the Morphogenesis program to apply it to a selection of basic morphogenetic processes. During the implementation, it became clear that some functionalities of the code were not yet ready for the realisation of gene regulatory mechanisms. In the given time frame, it was not possible to fix the code, and an alternative needed to be found. The decision to pre-model the morphogenetic processes in Python made it possible to avoid writing a monographic work about morphogenesis but provide the best possible groundwork with the limited possibilities at hand. The results gained from the Python scripts still need to be revised and expanded for an easier transfer to CUDA/OpenCL code. The most obvious and limiting factor of the Python script version compared to the CUDA model is the missing parallelisation, as all calculations are being performed on the CPU. However, this aspect can be ignored, as no more than a few hundred cells need to be simulated for the specific morphogenetic principles implemented. The provided implementations of the gene regulatory networks or the activation and inhibition mechanisms are potentially not directly applicable in the context of morphogenesis. Additionally, because the parameters were chosen manually, a sweep might provide a very different set of values with much more stable conditions.

The reason why the initial version of Morphogenesis was not directly written with OpenCL device code was that FluidsV3 (Rama Hoetzlein, 2012), which forms the technical core of the program, was written with CUDA device code. As it was planned to build upon the OpenCL-Version of Morphogenesis for this thesis, it should be mentioned why this translation is sensible and what changes need to be applied. The first reason for this conversion is the greater number of devices that would be able to edit, debug, and run Morphogenesis. OpenCL makes the program more accessible and portable for researchers. The second reason is that supercomputing clusters are starting to use non-Nvidia GPUs, making it practical to write platform-independent code. A plan has been devised to make the code run across multiple GPUs and cluster nodes using MPI, which is available on most clusters. The kernel compiler is determined by the OpenCL platform being used.

There are a few concrete changes that could be applied to Morphogenesis to enhance its value and efficiency as a tool and further improve the quality of the output.

1. Some improvement would be obtained by saving lists of particles in range, to use for each morphogen diffusion. The code is designed to make dense lists of particles for each active gene, each morphogen, solid vs fluid, and living vs. non-living particles. This allows only the necessary particles to be run for each of the kernels, which potentially saves a lot of computing time, making complex simulations tractable. The key concept is that the same could be applied to diffusion.
2. As mentioned, the translation of the CUDA-version of the code into a more broadly accessible and portable OpenCL-version would expand the code's possibilities. Besides that, as the program can also be used to model various soft-matter tissues and cellular systems that don't have to utilise the morphogenetic aspect of the program, it can also be used for its integration into physically grounded causal modelling.

The development and utilisation of Morphogenesis (Hockings & Young, 2021) represents significant strides in the simulation of complex developmental processes. It offers a controlled computational environment in which variables can be manipulated systematically and the extensive problem space of parameters can be explored more efficiently than with other experimental methods. The input data for these simulations enables the modelling of morphogenetic processes that occur at the intersection of genetics and intercellular mechanisms. The key benefits of the Morphogenesis simulator lie in its ability to investigate how minor changes in input parameters affect the system as a whole, and to model mutability for each gene. Stable parameter ranges can be efficiently identified through systematic parameter sweeps, which facilitate the development of robust and reliable simulations.

6 Conclusion

Despite the unforeseen technical challenges that prevented the full implementation of processes of embryonic development in the Morphogenesis simulator within the scope of this thesis, substantial progress was made to make the modelling approach more tractable. The theoretical groundwork gathered, and the Python scripts developed for this thesis provide the foundation for future practical implementations. The scripts mimic the underlying mathematical principles of diffusion and genetics to be used for more complex simulations once the Morphogenesis program is completed. Especially with the OpenCL-Version of the code, higher accessibility, and portability are enabled by a wider range of usable computational devices and supercomputing clusters. The utility would be maximised if in the future an MPI multi-GPU version is written. Of course, the importance and budget of a research project would need to be high to occupy large parts of a research cluster, but morphogenetics and disease processes are important fields of research.

Three processes of early development in vertebrates were chosen to be modelled, to offer a more hands-on perspective of the conceptual framework. The key idea behind their realisation was to take the current understanding of morphogenetic processes provided in the introduction and establish the computational backbone of the known principles of these dynamical systems of interacting morphogens while leaving out the mechanical forces. This makes a lot of sense for pole selection and FLS, but as it turned out, simulating somitogenesis with static cells compromises key functionalities of the process. Therefore, it would make more sense to model somitogenesis directly in Morphogenesis.

The amount of top-down and bottom-up approaches to morphogenesis is increasing. We explored many of them at different levels of detail. As mentioned at the beginning, the combination of both these categories will enable us to understand morphogenesis.

The major long-term goal of Morphogenesis is the complete simulation and thus understanding of genetic control over morphogenetic processes. The vision here is to be able to model any tissue *in silico* and enable morphogenetic design. Consequently, it would be possible to design “biological machines” to one’s liking. If a certain phenotype is needed, with Morphogenesis, or what will be built upon it, it will be possible to find the right gene regulatory mechanisms

affording the body plan. This, of course, is an outlook far into the future and it is important to focus on things that can be accomplished sooner.

The modelling of morphogenetic processes in general has many areas of application on the horizon. In essence, it allows for the realisation of gene regulatory networks in the form of interacting particles and their forces. Thus, every process that depends on a genetic basis but is ultimately realised in 3D space can be worth simulating. This can help to understand the complex mechanisms underlying different components of an organism not only during development but also during regeneration. One of the most important topics in this regard is tissue engineering. Growing tissue depends on a lot of factors influencing its health and stability. With the help of Morphogenesis, it would be possible to simulate these processes to improve the quality of *in vitro* methods. Other areas of application are genetic diseases, including the field of oncology, and the investigation of physiological and pathophysiological processes. In cognitive neuroscience, powerful phenomena to apply Morphogenesis to, are the emergence of consciousness, rationality and animal or human intelligence. The program could aid in finding a solution for the intricate development of the brain but also provide the necessary environment to expand our imagination on what biological intelligence is and what forms it can take.

In the short term, we would like to prove that a subset of known mechanisms of morphogenetics, and DNA mutation are sufficient to produce the complex, yet highly evolvable anatomy observed in biology. Mechanisms of artificial genomes developable with Morphogenesis will aid in discovering the true mechanisms in biological morphogenetics, which are only partially known. The program can also help test theories of tissue behaviour, for the study of development and disease processes, that resemble or interface with anatomy.

7 References

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Supplementary Material

The Python code used for this thesis can be found in this GitHub repository:

https://github.com/linusgoldgruber/thesis_supplementary_material