Mutual Inhibition Model of pattern formation: The role of Wnt-Dickkopf interactions in driving *Hydra* body axis formation

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Abstract

The antagonistic interplay between canonical Wnt signalling and Dickkopf (Dkk) molecules is fundamental to tissue organisation, including stem cell differentiation and body-axis formation. Disruptions in this interaction are linked to various human diseases, yet the mechanisms enabling robust body-axis formation through β-Catenin/Wnt-Dkk interactions remain unclear. A key model system for Wnt-driven pattern formation is the pre-bilaterian organism *Hydra*, where two ancestral Dkks interact with Wnt signalling to self-organise the body axis. While *Hydra* patterning has been extensively studied using the activator-inhibitor framework, a model integrating experimentally identified molecules has been lacking. Here, we introduce a mathematical model incorporating both Dkks and their experimentally observed interactions with Wnt signalling. Numerical and analytical studies show that this network alone is sufficient to drive *de novo* body-axis formation across a broad parameter range. Our mutual inhibition model offers an alternative to the classical activation/long-range inhibition paradigm, providing a mechanistic explanation for the observed Dkk and Wnt patterns under various conditions. Unlike previous models, it is directly grounded in experimental data, links injury response to pattern formation, and remains robust against perturbations.

Author Summary

How organisms form and regenerate complex body structures is a fundamental question in biology. In the freshwater animal *Hydra*, which can regenerate its entire body from a small tissue fragment, a molecular signalling system involving Wnt proteins and their inhibitors, the Dickkopf (Dkk) family, plays a central role in organising the body axis. While these molecules are known to interact, their exact roles and how they collectively shape large-scale patterns have remained unclear—especially since their activity does not fully align with established pattern formation models. In this study, we present a new mathematical model of reaction-diffusion-ODE type that captures the observed interactions between Wnt and two Dkk molecules in Hydra. We show that a mechanism based on mutual inhibition—rather than the traditional activator-inhibitor principle—can explain the emergence of a stable body axis and the results of various perturbation experiments. Our work offers new insights into the design principles of biological pattern formation and emphasizes the importance of exploring alternative mechanisms beyond classical theories.

Introduction

Cnidarians, with their simple body plans and remarkable regenerative abilities, offer a powerful model system for studying fundamental and broadly applicable principles of development and pattern formation [1–3]. Among them, *Hydra* has served as a classic organism in developmental biology for nearly 300 years, owing to its continuous morphogenetic activity, capacity for whole-body regeneration, and amenability to experimental manipulation [4–7]. In *Hydra*, body axis formation is a striking example of a self-organising process: even when dissociated into individual cells, aggregates can regenerate into functional polyps [8, 9]. This regeneration showcases *de novo* pattern formation, where cells determine their fate based on positional cues rather than retaining memory of their axial origin [10, 11].

To explain such processes, various mathematical models have been proposed. Many adopt a top-down, abstract approach to infer the interactions that could underlie observed patterns. A foundational concept comes from Alan Turing's theory of reaction-diffusion systems, where interacting morphogens produce patterns through diffusion-driven instabilities [12–14]. Building on this, Gierer and Meinhardt developed the activator-inhibitor model for *Hydra*, postulating that local self-enhancement of an activator, coupled with long-range inhibition, could account for head, foot, and tentacle patterning [15–17]. A later refinement introduced the concept of a slowly changing source density (SD), representing a memory of the body axis that stabilises and aligns spatial domains [18, 19].

From a molecular perspective, canonical Wnt/β-Catenin signalling governs posterior identity in many organisms, while inhibitors such as Dickkopf (Dkk) proteins define anterior fates by antagonising Wnt activity [20–24]. In vertebrates, Dkk proteins play essential roles in axial and head development and have been linked to various human diseases, including cancer and neurodegeneration [21, 25]. In *Hydra*, a similar antagonism between Wnt/β-Catenin signalling and *HyDkk* genes appears to underlie axis and head formation during regeneration [4, 5, 26–30]. Nuclear β-Catenin and expression of genes like *HyWnt3* mark the oral pole, while *HyDkk1/2/4-A* and *HyDkk1/2/4-C* are expressed in the body column and suppress Wnt signalling downstream [29, 30]. These genes are considered evolutionary precursors of vertebrate Dkk homologs [29].

However, while the activator-inhibitor model provides a compelling theoretical framework for *de novo* pattern formation, its alignment with known molecular pathways in *Hydra* remains problematic. Although canonical *HyWnt* signalling is a plausible candidate for the activator, a corresponding diffusible inhibitor that fits the model's assumptions has yet to be identified. Moreover, regeneration in *Hydra* often requires strong wound signals to initiate pattern formation, rather than emerging spontaneously from instability in a homogeneous field [19, 29, 31]. This challenges the model's central premise of self-organisation without external cues.

Expression patterns of *HyDkk* genes in *Hydra* deviate from the activator-inhibitor model predictions. *HyDkk1/2/4-A* is uniformly expressed, and *HyDkk1/2/4-C* decreases towards the aboral end—neither is expressed at the oral pole, contradicting the expected inhibitor gradient [29, 30]. Additionally, both genes are downregulated upon GSK3 inhibition, complicating their role in Wnt regulation and pattern formation [29, 30]. These discrepancies led to a reconsideration of their function, and the molecules were excluded as candidates for the self-organised pattern formation framework in *Hydra* [17, 32]. Consequently, research shifted towards identifying a missing inhibitor to fit the activator-inhibitor model, such as the transcription factor *Sp5*. Yet *Sp5* lacks diffusibility and fails to match predicted spatial expression profiles [33].

Other candidates, including thrombospondin (*TSP*) and the secreted protease *HAS-7*, have been proposed as Wnt antagonists [34, 35]. However, their expression patterns and functional effects do not conform to the spatial dynamics required by the classical model. For instance, *TSP* affects

tentacle patterning but not head or axis specification, while *HAS-7* appears to fine-tune local patterning rather than acting as a global inhibitory signal.

In light of these challenges, it seems increasingly unlikely that *Hydra* contains a molecular inhibitor that fully matches the assumptions of the classical activator-inhibitor model. Rather than seeking molecular players to fit an idealised framework, we propose reversing the approach: using a model-based analysis to explore whether the known interactions between *HyDkk1/2/4-A*, *HyDkk1/2/4-C*, and Wnt/β-Catenin signalling are sufficient to explain spatial patterning in *Hydra*.

The remainder of this paper is structured as follows. In the Results section, we introduce the new Mutual Inhibition (MI) Model that captures key molecular interactions between Wnt and Dkk proteins in *Hydra*. We present the model formulation, including biological justification and mathematical structure, followed by a comparison with experimental data and simulations of classical and novel perturbation scenarios. Next, we perform an in-depth mechanistic analysis for a reduced one-dimensional version of the model, using both numerical and analytical techniques to investigate conditions for pattern formation, including bistability and Turing instability. We explore the model's robustness to parameter variations and qualitative modifications. Technical details, extended model variants, and supporting analyses are provided in the Supporting Information.

Results

Mutual Inhibition (MI) Model

To explore pattern formation ability of the Wnt-Dkk signalling system, we propose a mechanistic model describing interactions of β-Catenin/Wnt and the two Dkk-molecules HyDkk1/2/4-A and HyDkk1/2/4-C in *Hydra*. An overview of the system components and their interactions derived from various experiments is given in Fig. 1 (simplified) and Fig. 2 A (in detail). The model is denoted as the *Mutual Inhibition (MI) Model* after its core feedback loops that stand behind the pattern forming mechanism. The aim of our approach is to determine if a specific structure of the Wnt-Dkk signalling network is sufficient to explain the formation of the body axis in *Hydra*. The choice of the model components and their interactions is motivated by their experimentally documented function during *Hydra* axis formation and regeneration [26–30].

Model equations of the signalling system and their biological justification

The core of the model is given by reaction-diffusion-type equations describing dynamics of five components, see Eq.(1)-(5) and Table 1. The model variable Wnt_{loc} represents the cell-local head-related molecules, e.g. reflected by the patterns of *HyWnt3, HyWnt9/10c, β-Catenin* and *TCF* expression [26–28]. The model variable Wnt_{diff} stands for the free Wnt ligands (such as ligands of HyWnt3 or HyWnt9/10c) [36]. Variables $DkkA$ and $DkkC$ describe HyDkk1/2/4-A and HyDkk1/2/4-C, respectively [29,30]. The model also incorporates the so-called source density SD, a slowly evolving long-term store of information about the body axis gradient. Although its molecular identity remains unknown, its existence has been demonstrated and plausibilised through various

experiments, see Refs. [4, 9, 15, 37–40] and more details below. The model equations read

$$\partial_t Wnt_{loc} = \frac{b_1 SD}{(1+k_1 DkkA)(1+k_2 DkkC)(1+k_3 Wnt_{loc})} - c_1 Wnt_{loc} \tag{1}$$

$$\partial_t DkkA = a_2 \Delta^\Gamma DkkA + \frac{b_2}{(1+k_4 Wnt_{loc})} - c_2 DkkA \tag{2}$$

$$\partial_t Wnt_{diff} = a_3 \Delta^\Gamma Wnt_{diff} + b_3 Wnt_{loc} SD - c_3 Wnt_{diff} \tag{3}$$

$$\partial_t DkkC = a_4 \Delta^\Gamma DkkC + \frac{b_4 Wnt_{diff}}{(1+k_5 Wnt_{loc})} - c_4 DkkC \tag{4}$$

$$\partial_t SD = a_5 \Delta^\Gamma SD + b_5 Wnt_{loc} - c_5 SD. \tag{5}$$

The choice of the domain is discussed later.

Wnt dynamics: Eq. (1) describes the cell-local canonical Wnt dynamics, denoted as Wnt_{loc}, as detailed above. The model accounts for both Wnt production and degradation, where production is induced by the source density SD, exhibits a saturation effect, and is inhibited by the two Dkks (denominators). In turn, diffusing Wnts (Wnt_{diff}) are described by Eq. (3). Its secretion depends on the SD and the cell-local Wnt_{loc}. Supporting experimental evidence is given in [26–28, 36, 41, 42]. However, we note that the diffusion strengths of Wnt molecules in *Hydra* remain unknown, making the assumption of relatively strong diffusion to a certain extent speculative. In this model, diffusion is essential for reproducing the domain of Dkk production, which is suppressed far beyond the expression area of e.g. *Wnt3* (only 5-15 cells [43]) and its inverse pattern coincides rather with nuclear TCF or β-catenin activity [26]. It matches the observation that *Wnt3* expression does not span the entire region of nuclear β-catenin activity, suggesting the involvement of an additional mechanism inhibiting Wnt3 production and restricting its expression domain. The existence of two feedback loops to explain this phenomenon was postulated in a recent study [44]. Here, we omit the fine-tuning of the Wnt3 and β-catenin patterning and focus on the dynamics of Wnt-Dkk interactions, while not splitting between Wnt3 and β-catenin; particularly since our model variables Wnt_{loc} and Wnt_{diff} represent various molecules, including several canonical Wnts with different activity ranges [36]. Additionally, recent studies suggest that mechanisms beyond chemical diffusion may complement or replace these long-range Wnt effects in *Hydra*, such as mechanical processes or properties [45].

Dkk dynamics: Eq. (2) describes the dynamics of HyDkk1/2/4-A, comprising of diffusion, degradation, and a production that is inhibited by Wnt_{loc} [29, 30]. Eq. (4) describes the dynamics of HyDkk1/2/4-C [30]. The key assumption on the mutual inhibition of HyDkk1/2/4-A and cell-local canonical Wnts follows the alsterpaullone (ALP) treatment experiments [29]. They demonstrated that *HyDkk1/2/4-A* expression in *Hydra* was negatively regulated by HyWnt3 and that removal of *HyDkk1/2/4-A*-expressing gland cells led to ectopic *HyWnt3*-expressing cell clusters throughout the body column. The inhibitory influence was additionally confirmed by analysis of the impact of *Hydra* HyDkk1/2/4-A on canonical Wnt signalling in *Xenopus* [29], and in another recent study showing that knockdown of *HyDkk1/2/4-A* in combination with ALP treatment led to the formation of ectopic axes in *Hydra* [35]. A similar mutual negative interplay has been suggested between HyDkk1/2/4-C and HyWnt3. ALP treatment induced a strong body-wide reduction in *HyDkk1/2/4-C* expression, and removal of *HyDkk1/2/4-C*-expressing gland cells led to an extension of *HyWnt3* expression in the head region [30]. Interestingly, in the presented model, the negative influence of canonical Wnt signalling on HyDkk1/2/4-C is not required for the general ability of patterning, but appears to strongly restrict the expression domain of canonical Wnt signalling (Supplementary Figure S 6 E–H).

The mechanistic difference between the model equations of HyDkk1/2/4-A and HyDkk1/2/4-C lies in the positive influence of Wnt signalling or its downstream products on the expression of *HyDkk1/2/4-C*. This was observed in co-expression studies and LiCl treatment [30], which are

Variable name	Explanation
Wnt_{loc}	cell-local head-related molecule complex comprising canonical Wnts, e.g. HyWnt3 and HyWnt9/10c (e.g., Ref. [26–28]).
$DkkA$	Dickkopf1/2/4-A - a Wnt antagonist in *Hydra* [29, 30].
Wnt_{diff}	free (secreted/diffusing) canonical HyWnts (with patterns corresponding to nuclear β-Catenin activity)
$DkkC$	Dickkopf1/2/4-C - a Wnt antagonist in *Hydra* [30]. Optionally augmented by the astacin protease HAS-7 [35].
SD	source density (SD) (also called e.g. *positional value* or *head forming competence*) - long-lasting information on the body-axis gradient; molecular/physical nature unknown.

Table 1. Model variables and their biological meanings.

known to manipulate source density and, hence, Wnt signalling in *Hydra* [39, 46]. Additionally, the observed ring pattern of *HyDkk1/2/4-C* expression suggests that the Wnt-related activation of HyDkk1/2/4-C and its inhibition must operate on different spatial scales, which may support the assumption of a positive influence from secreted HyWnt3 on *HyDkk1/2/4-C* expression. This assumption is consistent with the broader domain of nuclear β-catenin activity mentioned above, which extends beyond the *Wnt3* expression patterns. This could be addressed by an extended model that explicitly differentiates between β-catenin and HyWnt3 which we omit here [44]. It should be emphasised that although the model results are robust to changes in HyWnt diffusion (Supplementary Fig. S 6 A–D), the assumption of relatively fast diffusion remains meaningful. On the other hand, it can be verified that the simulated patterns do not critically depend on diffusion rates of HyDkk1/2/4-C and HyDkk1/2/4-A (Supporting Figure S 6 K-M).

Source density: Equation (5) describes the source density (SD) that provides a long-term memory of Wnt activity. The justification for this source density equation was already rooted in early grafting and regeneration experiments, which demonstrated that *Hydra* patterning involves two separate mechanisms acting at different temporal scales, referred to as *stable* versus *labile* head activation [37]. Indeed, ALP treatment, which blocks glycogen synthase kinase 3β and thus increases nuclear β-Catenin [28], indicated that the half-life of activated molecules (such as β-Catenin, HyWnt3, or Brachyury) ranges from hours to a maximum of 2-3 days. In contrast, body-axis gradient information, experimentally determined by the head formation capacity of grafted tissue pieces, lasted up to 6-8 days [17, 27, 42, 47]. This long-term storage of the body-axis gradient appears to be closely related to β-Catenin, despite the differing half-life. However, additional experiments show that it is not identical with β-Catenin: for example, during foot regeneration, β-Catenin is upregulated at the aboral pole [48], where head formation capacity is minimal [37]. Various experiments have demonstrated that SD is induced by high levels of HyWnt3/nuclear β-Catenin [27, 28]. In turn, head regeneration (and the HyWnt3/β-Catenin-mediated expression of head-related genes) always occurs at the most apical part of the Hydra body column [26, 36, 41, 42], indicating mutual activation between HyWnt3/β-Catenin signalling and the SD. For further details and motivations see Ref. [17, 18, 32, 44].

Model of the *Hydra* tissue

As the focus of this work is on the ability of the Wnt-Dkk signalling system to control the formation of a stable body axis, we investigate the proposed model both on a simplified one-dimensional domain representing the body axis ('1D model') and in a more realistic geometry of the *Hydra* tissue ('pseudo 3D model'). For the latter, we adopt a mechano-chemical modelling framework coupling the signalling system with a model of an infinitely thin deforming tissue [49–51]. Based on minimisation of the Helfrich free energy describing elastic tissue deformations, the model results in a 4th order partial differential equation of the tissue evolution governed by the gene expression patterns resulting

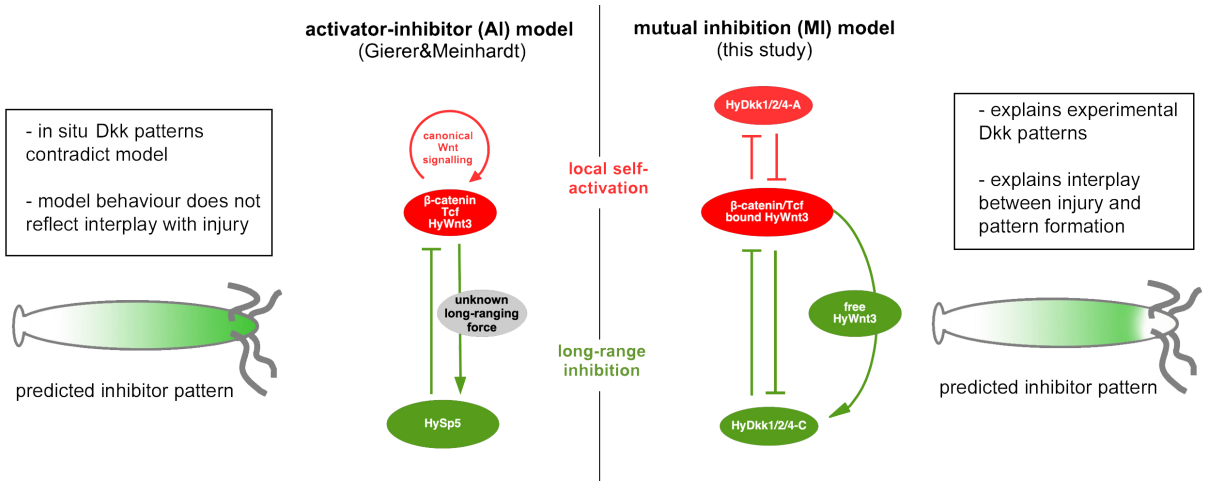

Fig 1. Schematic comparison of the activator-inhibitor model vs. the Mutual Inhibition (MI) Model. For the sake of clarity, in both models, the interplay with the source density and foot/tentacle patterning systems is not shown. Both models satisfy the general principle of local self-activation (corresponding components/interactions are shown in red) and long-range inhibition (corresponding components/interactions are shown in green). Grey ellipses represent still hypothetical components/mechanisms. Model limitations respectively improvements are listed within grey boxes. Canonical HyWnt signalling is not explicitly considered in the mutual inhibition model for the sake of complexity, but may depict an additional/redundant mechanism for local self-activation. Predicted expression patterns of the inhibitor differ between the models (sketched polyps); the mismatch between predicted and real HyDkk patterns has led to the neglect of HyDkk molecules in the activator-inhibitor model.

from the MI model. As this modelling approach is borrowed from previous works, we refer for details to Supporting Information. In contrast to the fully coupled mechano-chemical models of [45, 50, 51], the current model does not account for any feedback from the mechanical properties of the tissue to the gene expression processes. Consequently, the pattern formation process is induced by the chemical signalling system. The purpose of including a realistic geometry in the evolving domain was to examine the potential impact of the underlying geometry on the sensitivity of pattern formation dynamics. To facilitate model analysis, we simplified the system to a one-dimensional domain with zero-flux boundary conditions, which serves as a simplified representation of the *Hydra* body column. The reduction in complexity enabled the efficient execution of numerical simulations and facilitated a more tractable analysis of the pattern formation mechanism.

Model extensions to account for foot and tentacles dynamics

An additional version of the model includes two separate pattern-formation systems controlling foot and tentacle formation. These processes are not the focus of the study, as they do not contribute to the pattern formation mechanism of the body axis. However, implementation of the additional system components and their interplay with the core signalling system allows testing the model using additional experiments, such as e.g., the ALP experiments. Their dynamics contribute also to the realistic tissue geometry, i.e., a cigar-shaped body exhibiting local tissue evagination at the developing tentacles. Details of the foot/tentacle system, including experimental justification of the model are provided in the Supporting Information and in the references depicted in Fig. 2.

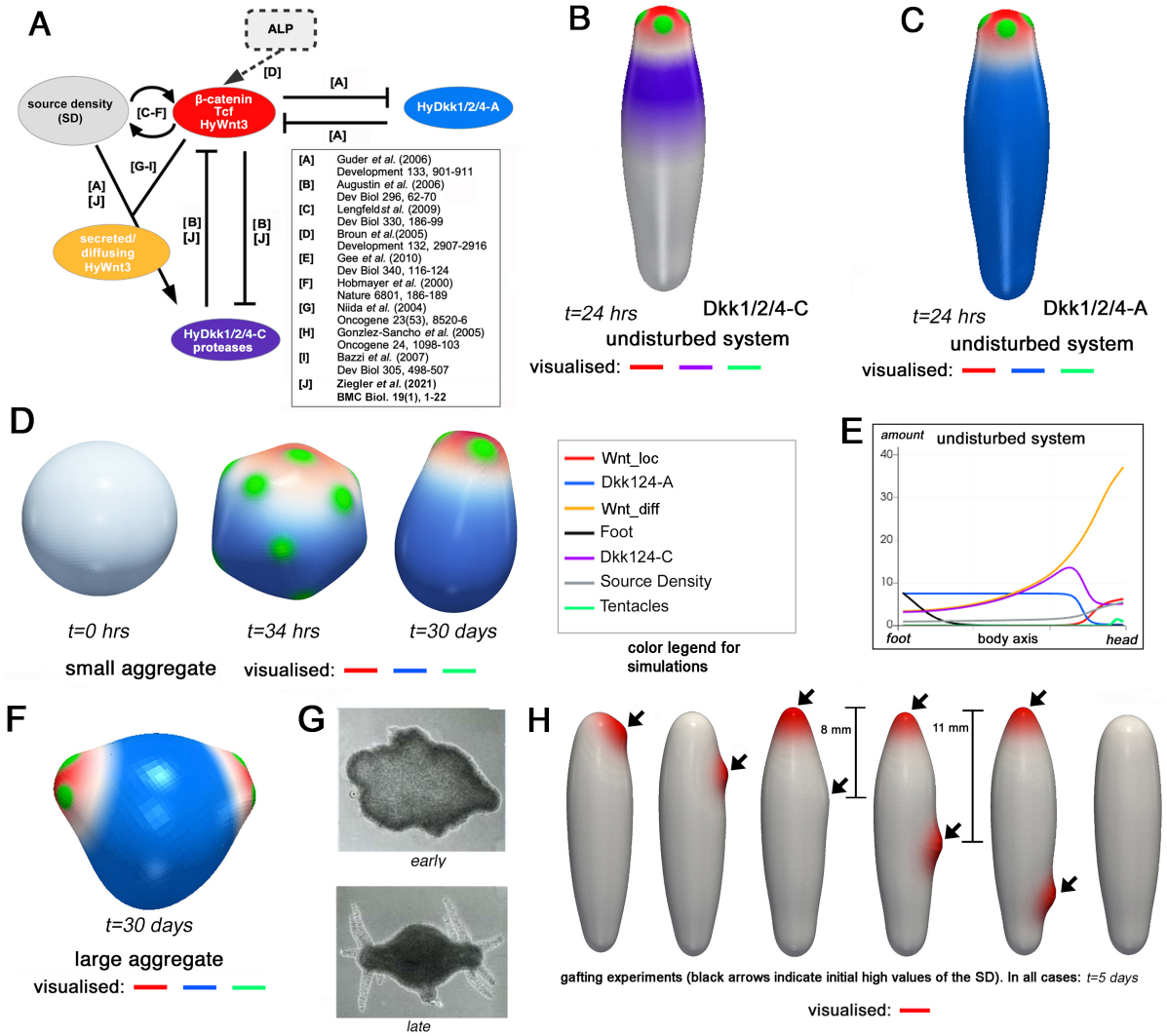

Fig 2. Overview, experiments, and simulations of HyWnt-Dkk-interactions and patterns. (A) Sketch of the *Hydra* body-axis formation network including main underlying references. For the sake of clarity, interactions with the tentacle and foot system are not shown. (B)-(C) Simulated expression patterns of HyDkk1/2/4-C (B) and HyDkk1/2/4-A (C) in the undisturbed polyp. (D) Different stages of a simulated small aggregate; (E) concentration profiles of the different variables along the body axis in the undisturbed system; (F) late stage of a simulated large aggregate vs. early and late stages of experimental large aggregate (G). (H) simulated head formation resulting from virtual grafting experiments. Black arrows indicate initially given high values of the SD representing grafts. Colour-scaling is similar in all simulation snapshots in this figure (c.f., color legend in the centre of the figure).

Model–Experiment Comparison and Validation

We evaluate the model's ability to replicate key experiments by presenting the outcomes of various simulations and comparing them to experimental results.

The MI model provides an explanation for the Dkk patterns that were not reproduced by previous models.

We start with numerical analysis of the wild-type pattern that resembles experimental observations (Fig. 2 B-C and Ref. [29, 30]). Such pattern can be established *de novo* if the initial conditions provide a sufficiently strong canonical Wnt signal localised at the head end, what corresponds to the head cutting experiments with a localised signal stemming from the injury. With respect to the resulting stable patterns, the simulations predict the lack of both HyDkk1/2/4-related molecules in the hypostome, showing a sharp expression border beneath the tentacles, *HyDkk1/2/4-C* expression fading out in the aboral direction (Fig. 2 B and E), and *HyDkk1/2/4-A* strongly expressed in the entire body column (Fig. 2 C,E). The simulated Wnt expression is restricted to the head region, fading within and below the tentacle zone (Fig. 2 B,C,E). Also, the temporal evolution of canonical Wnt activity matches qualitatively and quantitatively well between experimental observations and our simulations (Supplementary Figure S 7 F vs. Ref. [26]).

The difference in the two HyDkk patterns results from the qualitative differences in the corresponding production terms in the model equations. While HyDkk1/2/4-A is assumed to be constantly produced in the absence of repressing signals, HyDkk1/2/4-C production is modelled downstream of the HyWnt signalling and thus fades out in aboral direction. To explore the role of the constant production term, we additionally simulated the system with the HyDkk1/2/4-A production depending on SD instead of considering a constant expression. It led to a graded *HyDkk1/2/4-A* expression along the body axis fading out in ab-oral direction (Supplementary Figure S 6 I–J). In this modified system, the expression of Wnt_{loc} appeared to be distinctly increased in the budding zone (compared to the original model), indicating that body-wide expression of *HyDkk1/2/4-A* might be involved in controlling/suppressing bud formation.

It is important to notice that although both Dkk molecules are produced in overlapping domains and are based on mutual inhibition loops with canonical Wnt signalling, HyDkk1/2/4-A can be seen as a part of the local activation, whereas HyDkk1/2/4-C is rather involved in long-range inhibition (c.f., Fig. 1, Fig. 2, and Supplementary Fig. S 6 E–H). This apparent contradiction between antagonistic activity and similar expression patterns arises from the fact that the local activation is realised through an inhibition of the inhibitor HyDkk1/2/4-A (c.f., Fig. 1 and Fig. 2). Thus, the inverse expression pattern of HyDkk1/2/4-A is comparable to the activator pattern in the activator-inhibitor model and indeed, the inhibitory effect of HyDkk1/2/4-A on canonical Wnt signalling is required for pattern formation (Supplementary Figure S 7 E).

The MI model can describe self-organisation

To explore whether the observed ability of self-organised axis formation critically depends on the initially provided geometry or chemical (SD) gradient, we simulated self-organisation in *Hydra* aggregates. The experiment was modelled assuming a symmetric tissue sphere and a random distribution of all considered biochemical components, including the SD. The simulations showed *de novo* pattern formation resembling the experimentally observed patterns in adult polyps (Fig. 2 D,F,G). Notably, small initial spheres evolved into a single polyp (Fig. 2 D), and larger simulated aggregates developed multiple heads (Fig. 2 F), what matches well the experimental observations (Fig. 2 G). In conclusion, the MI model reproduces correctly the scaling of *de novo* pattern-formation system.

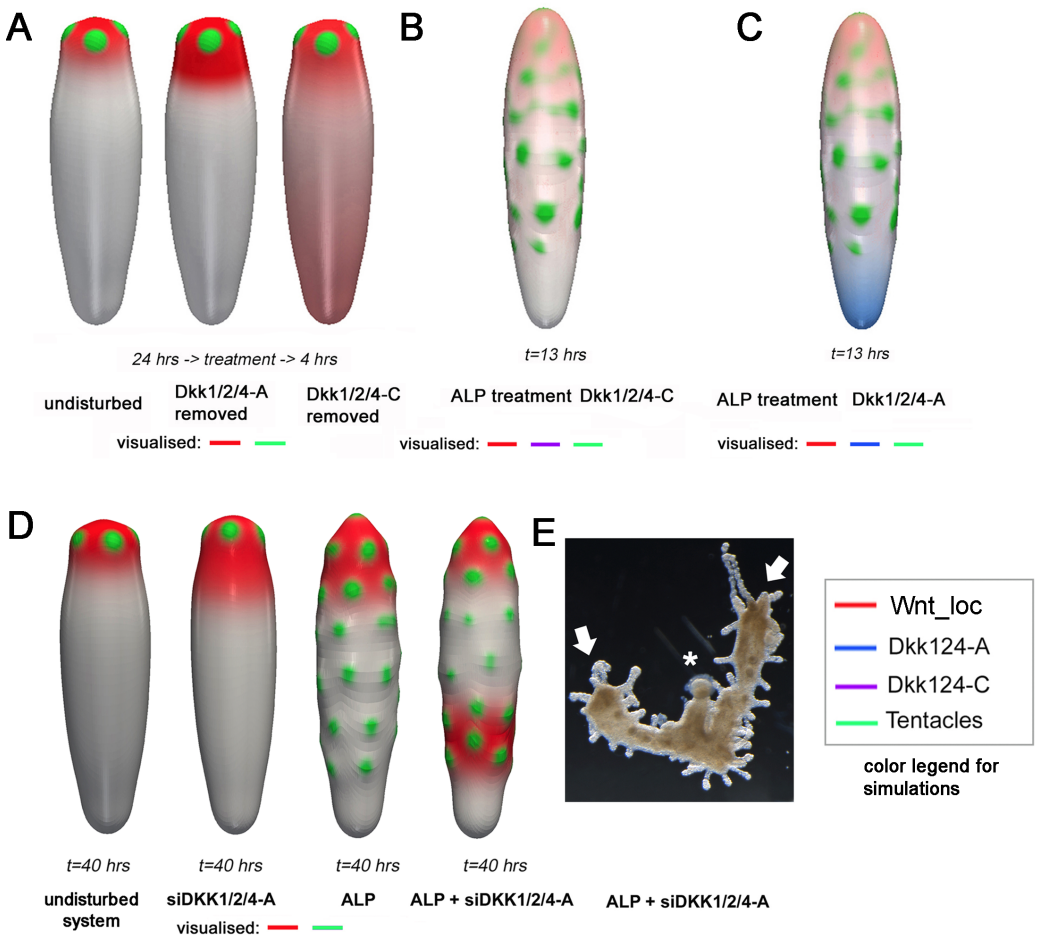

Fig 3. Simulations and experiments of manipulated HyWnt and HyDkk-levels. (A) simulation snapshots of the undisturbed polyp vs. virtual removal of HyDkk1/2/4-A vs. HyDkk1/2/4-C considering patterns of β-Catenin/TCF/bound HyWnt3 and tentacles. (B) Simulations of HyDkk1/2/4-A vs. HyDkk1/2/4-C expression after ALP-treatment. (D) Simulation snapshots of Wnt_{loc} and tentacle patterns of the undisturbed system vs. different combinations of ALP-treatment and HyDkk1/2/4-A knockdown. (E) experimental picture of double axis formation after ALP + siDkk1/2/4-A treatment, arrows indicate axes, asterisk represents foot.
Colour-scaling is similar in all simulation snapshots in this figure (c.f., colour legend on the bottom right-hand side).

The MI model requires a strong localised signal for head regeneration in cutting experiments

One of the key challenges to *Hydra* models of head regeneration is the recently demonstrated role of strong localised signalling in cutting experiments. These experiments show that removing the head without creating a wound does not lead to regeneration; only the activation of strong signalling and a localised increase in *Wnt3* expression as part of the wound response enables regeneration [19]. This finding is particularly significant as it directly challenges the classic *de novo* pattern formation mechanism described by activator-inhibitor models. In former theoretical work, we postulated the necessity of bi-stability in a model to account for such phenomena [52]. The MI model meets these conditions, describing the absence of pattern formation under certain initial conditions—specifically, when an area with high Wnt3 and source density levels is removed, Fig. 2 H – right hand side. Conversely, the introduction of a strong signal, such as that triggered by the wound response, facilitates pattern formation. A systematic analysis of this mechanism is provided in the next section.

The MI model reproduces the transplantation experiments

Further, we simulate transplantation experiments (Fig. 2 H) motivated by the seminal experiments presented in Ref. [37, 53, 54]. These experiments showed very early on that, under certain circumstances, tissue fragments transplanted from one polyp to the other can lead to the growth of a secondary body axis. Our simulations reproduce key properties. Head self-inhibition results in suppression of the head regeneration if two initiating signals are too close (3th simulation snapshot in Fig. 2 H). Moreover, without the head at the oral pole, grafts can induce a secondary axis at the arbitrary locations (1th and 2th simulation snapshot in Fig. 2 H). Simulations showed emergence of the secondary head in virtual graftings only when the distance is above approx. 50 % of the body length, which agrees with quantitative experimental observations [42] (4th and 5th simulation snapshot in Fig. 2 H). All these features of the *Hydra* patterning system stay in agreement with experimental observations [37, 42, 48, 54].

Further *in silico* experiments

In the next step, we verify the role of different model components by simulating their manipulated levels and comparing the resulting patterns to experimental data. First, we examine how system performance depends on the presence of the two HyDkk1/2/4-related molecules. Specifically, we simulate the undisturbed polyp system until the head pattern is established, as shown in Fig. 2 B,C and 3 A (left-hand side). We then perturb the system by virtually removing the expression of one of the two *HyDkks*. In line with experimental evidence, we observe a distinct expansion of Wnt_{loc} in the head region following the removal of HyDkk1/2/4-A (Fig. 3 A (middle) and Ref. [29]) and body-wide ectopic production of Wnt_{loc} after removing HyDkk1/2/4-C (Fig. 3 A, right-hand side as well as Ref. [30]). The only difference between the simulations and experiments is that Wnt_{loc} production appears relatively diffuse and homogeneous after reducing the number of HyDkk1/2/4-C-expressing cells (Fig. 3 A, third snapshot), whereas experiments report a more spotty pattern of *HyWnt3* expression [30]. This discrepancy is likely due to the fact that, as mentioned earlier, the model does not distinguish between the cell-local HyWnt3 and β-catenin or TCF, whereas in reality, these factors may belong to two distinct pattern-formation systems operating at different spatial scales [44]. However, for the sake of model simplicity, further distinctions between β-catenin-driven axis formation and HyWnt3-driven head/organisation formation (cf. [44]) are not considered in this study, as the primary focus is on the general principles of HyWnt-Dkk-driven axis formation.

In addition, we simulate the activation of canonical HyWnt signalling via ALP treatment (Fig. 3 B,C, as well as Supplementary Figure S 7 A–D). Experimentally, both HyDkks are suppressed by this treatment, along with the development of ectopic tentacles along the body column [29, 30]. However, *HyDkk1/2/4-C* expression appears to be more sensitive to β-catenin/HyWnt3 levels, as

the reduction in HyDkk1/2/4-A occurs later than the reduction in HyDkk1/2/4-C [29, 30]. These observations align with our simulation results during ectopic tentacle development (Fig. 3 B,C). However, we note that in later stages of the simulations, Dickkopf patterns re-establish (1D concentration profiles in Supplementary Figure S 7 A–D), suggesting that the model can reproduce the data only as transient patterns. This may suggest that ALP-driven effects are initially present but reversible. Finally, we simulate recent HyDkk1/2/4-A knockdown experiments [35] with and without ALP treatment (Fig. 3 D). Similar to the experimental results (Fig. 3 E), the combination of ALP treatment and HyDkk1/2/4-A knockdown leads to the development of a secondary ectopic axis, marked by an additional region with strong expression of the canonical HyWnt signalling complex in our simulation results, but again, this is observed as a transient pattern.

Analysis of the Mechanism Underlying Pattern Formation

To determine under which conditions the MI system can exhibit symmetry breaking and pattern formation, we analyse the structure and stability of spatially homogeneous steady states. Stability in these states indicates the absence of pattern formation in their vicinity, whereas a loss of stability—triggered by contrasting diffusion parameters (the diffusion-driven instability) can give rise to the well-known Turing pattern formation. For linearised and nonlinear stability analysis theory for reaction-diffusion-ODE models we refer to [55, 56].

Additionally, systems coupling diffusive and non-diffusive components can exhibit *far-from-equilibrium* patterns characterised by jump-discontinuity [57–59]. Such solutions emerge due to the system's bistability. While a Turing instability may act as a trigger, the emergence of *far-from-equilibrium* patterns can occur independently of the Turing mechanism. To better understand the nature of the patterns observed in our simulations, we analyse the stability of branching stationary solutions near the DDI bifurcation point.

Since a rigorous analysis of complex models is often infeasible, we complement it with numerical analysis. Guided by analytical insights, we analyse the model for various fixed parameter values. This is further supported by a sensitivity analysis, which assesses the model's robustness to parameter and nonlinearity variations and confirms the validity of the results within specific parameter regimes.

One-dimensional model reduction

In this section, we focus on a one-dimensional version of the model given by equation (1)-(5) to systematically analyse its dynamics as a function of the parameters and initial conditions. Our goal is to provide a deeper understanding of the simulation results discussed earlier. We begin by examining the model's ability to generate patterns. This involves analysing the mathematical structure of the model, the ability of symmetry breaking and the stability of the emerging patterns.

The reduced model is obtained by approximating the complex domain of *Hydra* by an interval $[0, 1]$ and rescaling time and model variables in equations (1)-(5). The technical details of the model reduction, analytical approach, and numerical implementation are provided in the Supporting Information. To compare the reduced system with the original full model, we apply a computational fitting procedure using data derived from the 3D simulation (Fig. 2 E). These data are obtained by averaging along the axis perpendicular to the body axis, followed by min–max normalisation. Parameter estimates are identified by minimising the least-squares residual between the data profiles and the output of the reduced model. This procedure demonstrates that the reduced model reproduces the same dynamics as the original, with appropriate parameter adjustments. Further numerical details are included in the Supporting Information Table S 1.

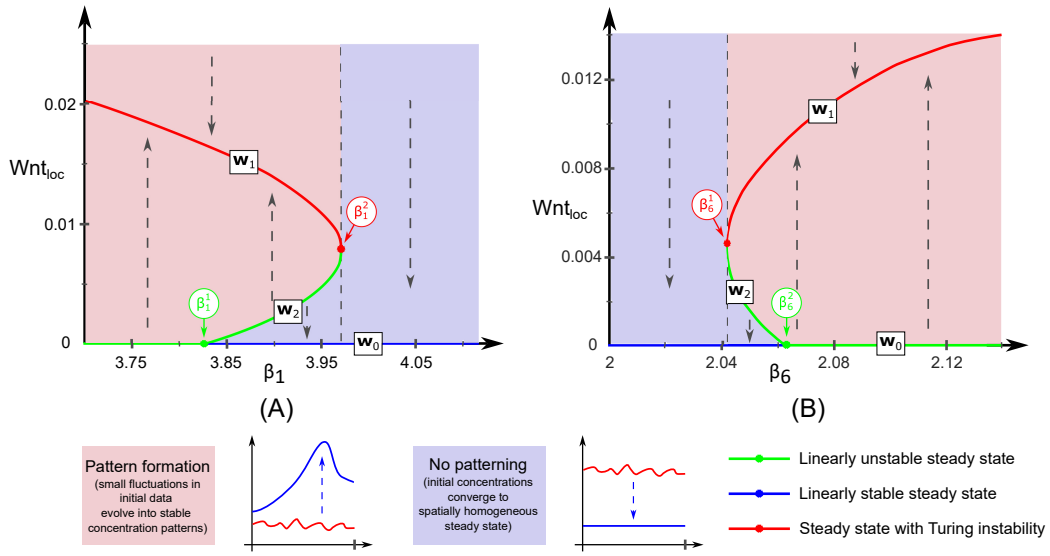

Fig 4. Bifurcation diagram illustrating the qualitative changes in pattern formation in the one-dimensional version of model (1)–(5), induced by variations in the rescaled reaction parameters $\beta_1 := b_2/c_2$ (effective production rate of $DkkA$) and $\beta_6 := b_1/(c_1c_5)$ (effective production rate of Wnt_{loc}). The vertical axis represents the first component of the homogeneous steady states. Arrows indicate whether initial conditions converge to the pattern formation regime or decay towards the trivial steady state. Coloured bullets mark the boundaries of the bistable region: β_1^1 and β_1^2 in panel (A); β_6^1 and β_6^2 in panel (B). All other model parameters are set to the values listed in Supplementary Tab. S 1.

The MI model reveals bistable behaviour in uniform steady-state structures

Analysis of the structure of the spatially uniform steady states demonstrates a bifurcation from a semi-trivial state ($Wnt_{loc} = 0, DkkA = \beta_1, Wnt_{diff} = 0, DkkC = 0, SD = 0$), where $\beta_1 = b_2/c_2$, resulting in the existence of additional spatially homogeneous solutions and a change of stability, see Figure 4. We identify two complementary bifurcation parameters, $\beta_1 := b_2/c_2$ and $\beta_6 := b_1/(c_1c_5)$, which describe the effective production rates of Dkk_A and Wnt_{loc}, respectively. More precisely, linear stability analysis of the semi-trivial steady state yields an instability condition, $\beta_6 > 1 + \beta_1$, which marks the bifurcation point and highlights the complementary relationship between the two bifurcation parameters. The corresponding bifurcation diagrams are shown in Fig. 4 A and Fig. 4 B. The remaining parameter values are fixed according to the parameter fit to the three-dimensional model discussed above (see Supplementary Table S 1).

The structure of the bifurcation diagram and the stability of different branches of spatially uniform solutions are determined based on the model analysis presented in the Supplementary Information (Lemma S1-S4 and Corollary S1) and numerical computations, employed whenever a complete analytical understanding was not feasible. To this end, we investigate the model for varying either β_1 or β_6, while fixing all other parameters. As theoretically predicted, for sufficiently small values of β_1, we observe instability of the semi-trivial stationary solution, denoted by $\boldsymbol{w}_0$, and existence of exactly one non-trivial spatially uniform steady state $\boldsymbol{w}_1$ which is stable for the system without diffusion. Numerical computation of the system linearised at this positive steady state indicates Turing instability. This stays in agreement with the model simulations showing globally stable pattern formation. Above the critical value (transcritical bifurcation point), the semi-trivial state becomes stable and gives branching to an unstable homogeneous steady state $\boldsymbol{w}_2$ to eventually collide with $\boldsymbol{w}_1$ for the parameter value corresponding to the saddle-node bifurcation point. This results in a bistable regime for the parameter $\beta_1^1 < \beta_1 < \beta_1^2$. This means that for initial concentration values

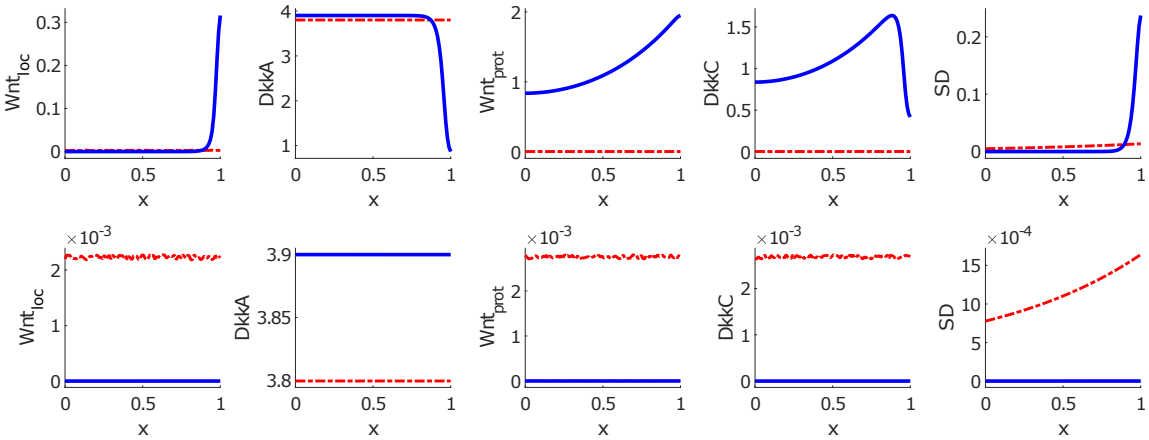

Fig 5. Example of bistable behaviour in the one-dimensional reduced model. Initial data are taken as small perturbations of the homogeneous steady state $\boldsymbol{w}_1$. When small perturbations lie in the basin of attraction of $\boldsymbol{w}_1$, pattern formation occurs and initial data evolves to the steady-state patterns (first row). Otherwise, when the steady state is perturbed in the *direction* of the trivial steady state $\boldsymbol{w}_0$, no pattern formation occurs and initial concentrations decay to the trivial steady state (second row). Parameter values used in the simulations are given in Supplementary Table S 1. Blue colour shows the final concentration profiles, while red colour denotes the initial concentration values.

in the basin of attraction of the trivial steady state $\boldsymbol{w}_0$, there is no pattern formation, and initial data decay to the trivial steady state $\boldsymbol{w}_0$. On the other hand, if initial data lie in the basin of attraction of the steady state $\boldsymbol{w}_1$, then we observe the formation of patterns. Here, unstable steady state $\boldsymbol{w}_2$ acts like a separation hyperplane between two regimes, see Figure 5. For $\beta_1 > \beta_1^2$ no pattern formation occurs and the only existing spatially uniform steady state $\boldsymbol{w}_0$ becomes a stable attractor. Analogous results are obtained with respect to the bifurcation parameter β_6, as shown in the bifurcation diagram Fig. 4 B.

Translated into the language of experimental research, this means that pattern formation or regeneration does not always occur as predicted by the classical activator-inhibitor model, but can critically depend on the initial conditions. This, in turn, explains why the MI model aligns with the experimental observation that regeneration only happens when the initial conditions provide a sufficiently strong stimulus through wound signalling.

MI model exhibits Turing pattern formation

The Turing instability leads to spatially heterogeneous solutions that may converge to stationary patterns. To gain a deeper understanding of the emerging patterns, particularly in the bistable region, we conduct a bifurcation analysis of branching solutions near the onset of Turing instability, see Supporting Information for details. Since not all required expressions can be derived explicitly, we compute certain terms numerically. We examine two sets of model parameters to encompass both regimes—one with hysteresis and one without—and use the largest diffusion coefficient as the bifurcation parameter. We then numerically compute the critical value and apply classical bifurcation theory to construct asymptotic expressions for branching stationary solutions and assess their linear stability. In both cases, our analysis indicates that the branching solutions remain stable, consistent with numerical simulations for the corresponding parameter values. Although our approach does not constitute a rigorous mathematical proof, it provides strong evidence supporting the presence of Turing patterns.

MI model is robust with respect to parameters' perturbations

Next, we numerically investigate the robustness of pattern formation with respect to variations in model parameters. To demonstrate this robustness, we use the parameter values listed in Supplementary Table S 1. We then explore the hypercube in parameter space for rescaled reaction rates β_i within a large range $[\beta_i/100, 100\,\beta_i]$, parameters are therefore varied by a factor of 10,000. To estimate the regions within this hypercube where pattern formation occurs, we employ Markov Chain Monte Carlo (MCMC) methods. The criterion for admissibility is the existence of at least one homogeneous steady state exhibiting Turing instability. For the chosen parameter values, we did not identify any cases where this criterion was not met, indicating the robustness of the observed pattern formation. Of course, the range of parameters studied here did not exceed the previously discussed points of transcritical bifurcation, beyond which the only stable equilibrium state of the system would be the semi-trivial state. Another critical parameter is, as discussed before, the largest diffusion in the system, which has already been discussed earlier and must be sufficiently large in order to achieve Turing instability.

MI model is robust with respect to a range of qualitative changes

Finally, we assess the robustness of the reduced model (for equations, cf. Supporting Information) with respect to modifications in the reaction terms. We consider two variations of the model. In the first, the reaction terms are multiplied by their respective denominator expressions. This modification can be interpreted as describing inhibition at the level of degradation or receptor binding, rather than at the level of production. Another system's perturbation concerns using higher-order terms in Hill functions, i.e. modulating the inhibition process. For the parameter values examined, both variations exhibit the same qualitative pattern formation behaviour as the original one-dimensional model, suggesting its robustness. For further technical details, see the Supporting Information.

Discussion

Symmetry breaking and pattern formation in *Hydra* have been extensively studied through the activator-inhibitor model, which has provided significant insights into the underlying processes [17, 60], and identified the pattern formation principle of local activation and long-range inhibition (LALI) [13, 32]. However, discrepancies have emerged between predicted and observed patterns, highlighting the limitations of the classical activator-inhibitor framework. Main challenges are: (1) the elusive identification of inhibitors consistent with the theory [45]; (2) observed patterns of key Wnt inhibitors, such as Dkk, contradicting the activator-inhibitor model [29, 30]; (3) the failure of the model to predict the necessity of wound healing signals for regeneration [19]; and (4) the requirement for specific nonlinear kinetics, which are difficult to justify from molecular or experimental perspectives [13, 61, 62].

To address these challenges, we developed the Mutual Inhibition (MI) Model, which: (1) is based on known Dickkopf inhibitors and their experimentally determined interactions; (2) generates patterns that align with experimental data for all required molecules, including both Dickkopfs; (3) produces spatial patterns only when initial conditions are sufficiently far from equilibrium, consistent with wound signalling; and (4) remains robust to parameter disturbances and qualitative changes. The MI model was validated through its alignment with a broad range of experimental data and manipulations, including genetic and pharmacological treatments and classical grafting experiments.

The new model provides insights into how Dkk molecules and canonical Wnt signalling may lead to self-organised pattern formation in *Hydra*, specifically through repeated motifs of mutual inhibition, realising the LALI principle. Although the Dkk-Wnt interplay is essential for various developmental processes [21, 63], the underlying patterning mechanisms have remained unclear. The

MI mechanism presented here may represent a general and fundamental patterning motif, extending beyond the phylum Cnidaria. However, a sufficiently strong diffusion for (some) Wnt molecules is still an critical assumption in the presented MI model. While studies in other organisms indeed suggest such diffusion [64–66], direct evidence in *Hydra* is still lacking. The assumed diffusion may also be interpreted as an effective measure for various propagation mechanisms, including the synergistic spread of multiple Wnts [36], biomechanical cues [67], bio-electrical signalling [68], or active transport along cell protrusions [69].

Beyond the biological insights, the study proposes a paradigm shift in pattern formation research. Traditionally, the top-down approach dominated, where theoretical models guided the search for matching molecular components. However, as recent advancements show a plethora of two- and three-component networks capable of self-organisation into Turing-like patterns [62, 70], a top-down approach may be inefficient. The present study adopts a bottom-up approach, building a model based on experimentally identified system components and interactions. By iteratively combining this approach with theoretical studies, we can develop effective models and generate specific questions for hypothesis-driven experimental research.

Additionally, the present study does not distinguish between body axis and head formation in *Hydra*, although recent findings suggest that these processes should be analysed separately [44]. The MI mechanism is more closely associated with the formation of the body axis, as the observed patterns establish on a large (body axis) scale rather than on the smaller scale of the head organizer. This suggests that other canonical Wnts, such as *HyWnt9/10c*, may play a central role in the activation process, as indicated by its early expression during regeneration [36]. Indeed, a *HyWnt9/10c* knockdown results in complete regeneration failure, while *HyWnt3* knockdown still allows tentacle formation, indicating that the body axis remains intact [19].

In the present study, various components such as β-Catenin, HyTCF, *HyWnt9/10c*, and *HyWnt3* were grouped into one molecular class, further distinguishing between cell-local and long-range components. This categorisation highlights several gaps, such as the broader spatial domain of β-catenin's nuclear activity compared to Wnt3. This suggests that canonical Wnt signalling may involve an additional mechanism to restrict Wnt3 patterns [44]. A more detailed view of these components in interaction with Dkk molecules in *Hydra* is needed to better distinguish between head and body axis formation. Further research could investigate the effects of *Dkk* overexpression or knockdown on the distribution and levels of these Wnt molecules.

In summary, over the last decade, our understanding of *Hydra* pattern formation has shifted significantly. Early models provided a basic framework but failed to account for the complex realities of pattern formation. The identification of distinct patterning loops and the recognition of non-chemical processes, such as cytoskeleton organisation and bio-electrical signalling, have added layers of complexity [45, 68, 71–75]. Moreover, the role of Wnt antagonists in regulation has revealed a more intricate picture than initially understood [29, 30, 33, 35]. The MI model presented here has allowed for a better exploration of different aspects of Wnt signalling that indicated gaps in the current systematic understanding of pattern formation in *Hydra*. The next challenge will be to integrate these findings into new experimental design to gain a coherent understanding of developmental patterning.

The MI model is also of significant theoretical interest, as it exemplifies a system that couples diffusing and cell-local components. Such reaction-diffusion-ODE models have been the focus of analytical research in recent years, as they can exhibit dynamics that extend beyond classical Turing systems [76–81]. Notably, in models where cell-local components interact with a single diffusing morphogen, all Turing patterns may become unstable [55, 82], giving rise to *far-from-equilibrium* patterns characterised by jump discontinuities [57–59]. However, models that couple space-dependent ODEs with multiple diffusive components, such as the MI model, remain largely unexplored. Our findings

demonstrate that in such models, stable Turing patterns can still emerge, even in conjunction with bistability, highlighting the need for further theoretical investigation.

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