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# Modeling Mammary Gland Morphogenesis as a Reaction-Diffusion Process

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*Abstract*- **Mammary ducts are formed through a process of branching morphogenesis. We present results of experiments using a simulation model of this process, and discuss their implications for understanding mammary duct extension and bifurcation. The model is a cellular automaton approximation of a reaction-diffusion process in which matrix metalloproteinases represent the activator, inhibitors of matrix metalloproteinases represent the inhibitor, and growth factors serve as a substrate. We compare results from the simulation model with those from in-vivo experiments as part of an assessment of whether duct extension and bifurcation during morphogenesis may be a consequence of a reaction-diffusion mechanism mediated by MMPs and TIMPs.**

*Keywords*—**Branching morphogenesis, simulation, modeling, mammary gland, extracellular matrix, reaction-diffusion, matrix metalloproteinase, tissue inhibitor of metalloproteinase.**

## I. Introduction

Branching morphogenesis occurs in the development of a variety of organs, from the trachea in Drosophila to nephrons in the kidney. Patterns in branching morphogenesis have been identified and include branch elongation, tip bifurcation, lateral branching, and anastomosis. Elongation and tip bifurcation both occur during mammary branching morphogenesis. What are the key factors and events? To help answer that question and gain a deeper insight into how such processes can unfold (and be influenced by interventions), we developed a cellular automata (CA) model and used it for experimentation. Here we focus on two classes of factors (defined below): MMPs alter the environment of the developing cells, and TIMPs specifically inhibit them. Our hypothesis is that they can work synergistically as activators and inhibitors in a reaction-diffusion process that can guide and control simulated branching morphogenesis.

## II. Biological Details

The formation of mammary ducts in the mouse also involves branching morphogenesis. It occurs in several stages, beginning in early development with the formation of the mammary bud at mid-gestation, followed by invasion of the epithelium into the mammary fat pad during puberty. During invasion, a branched ductal network is formed through a process involving extracellular matrix (ECM) remodeling, duct elongation, and branching. The process is facilitated by crosstalk between the proliferating epithelia and the mammary stroma. It has been demonstrated that mammary branching morphogenesis is influenced by extracellular matrix composition, growth factors, ECM-degrading enzymes including matrix metalloproteinases (MMPs), and tissue inhibitors of MMPs (TIMPs) [1] (Fig. 1). The matrix metalloproteinases catalyze the cleavage of extracellular matrix components, which is required for duct extension. Treatment of mice with broad-spectrum MMP inhibitors inhibits branching and duct elongation [2]. Tissue inhibitors of metalloproteinases serve to regulate the activities of matrix metalloproteinases, inhibiting MMP activity by noncovalent binding. It has been hypothesized that it is the coordinated action of MMPs and TIMPs that give rise to branched growth (Fig. 1B).

A number of approaches have been taken to model morphogenesis in biological systems. In particular,

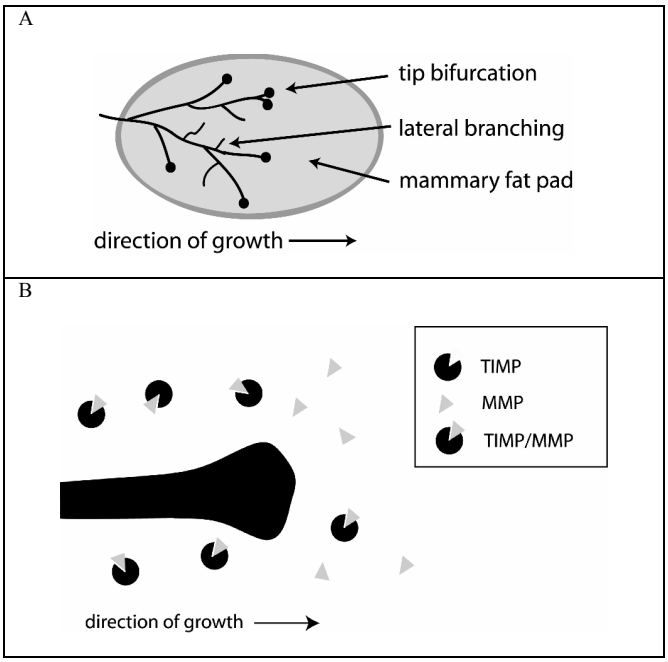



Fig. 1. A. Representation of branching morphogenesis in the developing mouse mammary gland. Black lines represent ductal cells, with each duct terminated by a terminal end bud, where duct bifurcation occurs. Development of the mature gland involves duct elongation, tip bifurcation, and lateral branching. B. Representation of inhibition of MMP action by TIMP expression.

reaction-diffusion systems have been used extensively in modeling pattern formation, including branching morphogenesis [3]. A reaction-diffusion system of the substrate-depletion type was used to model the influence of fibroblast growth factor 1 (FGF1) on the growth of lung epithelial cultures in tissue culture, where FGF1 served the role of the substrate, and cells served as the activator [4]. It has also been demonstrated that a reaction-diffusion system of the activator-inhibitor type is able to give rise to fundamental branching features such as bifurcation and lateral branching [5].

In the case of mammary branching morphogenesis, it is possible that MMPs and TIMPs may be acting as activators and inhibitors, respectively, in a reaction-diffusion process. It is known that MMPs exhibit autocatalytic activation. It is also known that MMP and TIMP expression is often co-localized. And finally, TIMPs are capable of inhibiting MMP activity in a 1:1 stoichiometric ratio. We have implemented a computer simulation of a reaction-diffusion process of branching morphogenesis adapted from the work in [5]. We compare results obtained from in-vivo experiments modulating MMP and TIMP activity with output generated from the simulation in order to explore whether MMPs and TIMPs may be functioning as activators and inhibitors in a reaction-diffusion process.

## III. Modeling and Simulation

The simulation is implemented as a 2D square grid cellular automata with closed boundaries. Four variables determine the state of each grid position in the cellular automata: matrix metalloproteinases (*MMP*)[1], tissue inhibitor of metalloproteinases (*TIMP*), growth factor (*GF*), and duct cells (*CELL*). The following is a description of each variable and the CA update rules which govern their behavior. The relationships between the variables are represented visually in Fig. 2, and the rules that govern the CA are summarized in Fig. 3.

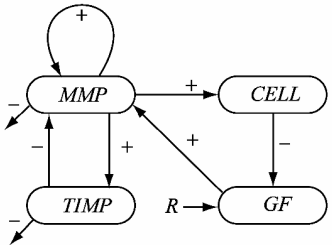



Fig. 2. Diagram of the relationships between the variables in the model, adapted from [5]. The dashed arrows are used to indicate whether diffusion occurs. *MMP*: stands for the variable representing matrix metalloproteinase levels; *TIMP*: stands for the variable representing tissue inhibitor of metalloproteinase; *ECM*: stands for the variable representing extracellular matrix; *CELL*: stands for the variable that indicates the presence of a simulated duct cell; and *R*: A small random variation in the value of *GF*.

[1] To avoid confusion, the simulation variables which correspond to biological features are italicized.

### A. *Metalloproteinases*

MMPs are proteases that degrade components of the extracellular matrix, particularly collagen and laminin. Most matrix metalloproteinases are secreted as proenzymes, and through autocatalysis they become activated. It is known that MMPs are selectively expressed around sites of duct elongation, and it has been demonstrated that MMP activity is required for normal mammary gland development [2]. It is also known that growth factors can induce expression of MMPs [6]. The ability of MMP to induce TIMP expression has not been verified in the literature; however, MMP and TIMP expression is often colocalized in-vivo, suggesting such a mechanism may exist. These behaviors are represented and controlled within the model by rules 1, 2, 3, 4, 7, 9, and 11.

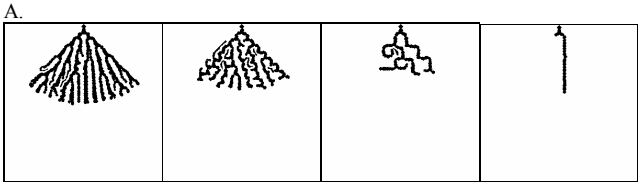
### B. *Tissue Inhibitors of Metalloproteinases*

Tissue inhibitors of metalloproteinases (TIMPs) are the primary regulators of MMP activity. The TIMPs have also been implicated in mammary branching morphogenesis [7]. One function of TIMPs is to bind to MMPs and inhibit their

1. If $MMP > d \cdot TIMP$, then $MMP = c \cdot MMP \cdot GF$
2. If $MMP > MMP_{\max}$, then $MMP = MMP_{\max}$
3. $MMP = d_1 \cdot MMP - d_2$
4. If $MMP < 0$, then $MMP = 0$
5. $TIMP = d_3 \cdot TIMP - d_4$
6. If $TIMP < 0$, then $TIMP = 0$
7. $TIMP = g \cdot MMP + TIMP$
8. $TIMP = TIMP + h$
9. If $MMP > e$, then $CELL = 1$
10. If $CELL = 1$, then $GF = GF + a - b_1 \cdot GF$, else $GF = GF + a - b_0 \cdot GF$
11. $MMP = \langle MMP \rangle$, $TIMP = \langle TIMP \rangle$, $GF = \langle GF \rangle$
12. $GF = GF + r\,N(0,1)$

Fig. 3. The CA transition rules used in the simulation, adapted from [5]. Default parameterizations: $a = 0.2$, $b_0 = 0.2$, $b_1 = 1.0$, $b_2 = 1.0$, $d_1 = 0.9$, $d_2 = 1.0$, $d_3 = 0.5$, $d_4 = 0.5$, $r = 0.01$, $\gamma = 0.5$, $\varepsilon = 120$, $c = 3.0$, $d = 2.0$, $MMP_{\max} = 250$, grid height = 300, and grid width = 300. Initial *GF* in every cell was set to 0.75, whereas initial *MMP*, initial *CELL*, and initial *TIMP* in every cell were set to zero. The simulation is triggered using a positive value of *MMP* placed in the corner of the simulation grid. The distances used for the averaging of Moore neighborhoods in rule 12 were $R_{MMP} = 2$, $R_{GF} = 3$, and $R_{TIMP} = 6$.

A.



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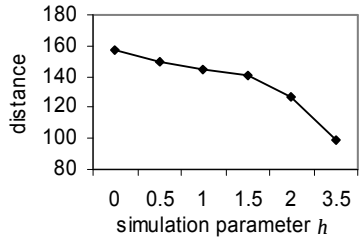


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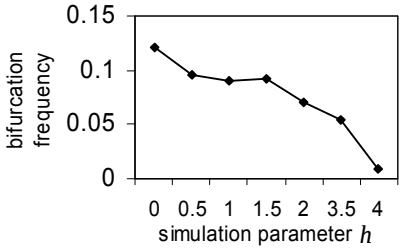



Fig. 4. Effect of increasing basal *TIMP* level, $h$, on simulation output. A. Simulation output with default parameter values from Fig. 2, but with $h = 0$, $h = 2.0$, $h = 3.5$, and $h = 4.0$, from left to right. B. Simulated duct extension distance for different values of $h$, after 300 steps, measured from the initiation point to the tip of the furthest simulated duct, in points. C. The effect of $h$ on the number of bifurcations per unit distance after 300 steps.

protease activity. The inhibitory activity of TIMPs is modeled in rule 1. Regulation of TIMP expression is modeled by rules 6 and 7. In order to imitate experiments in which TIMP is expressed constitutively, rule 8 has been included the model. The diffusion of TIMPs is governed by rule 11.

C. *Growth Factor*

It has been demonstrated in 3D cultures as well as in-vivo that growth factors can influence mammary branching morphogenesis [8]. In addition to growth factors such as epidermal growth factor, hepatocyte growth factor, and fibroblast growth factor, byproducts of extracellular matrix decomposition can stimulate branching. Growth factors are able to diffuse through the mammary fat pad, as indicated by studies of the effects of growth factor implants on duct growth in-vivo [8]. It is also known that growth factors can induce MMP synthesis [4]. Rules 1, 10, and 11 model these behaviors in the simulation. A small stochastic component is introduced into the model to model in-vivo heterogeneity in the mammary fat pad by rule 12.

D. *Cell Proliferation*

Cell proliferation occurs as a consequence of MMP activity. It is known that MMP digestion of ECM components generates growth-promoting fragments; furthermore, MMPs are able to degrade cell-ECM contacts, which can induce an epithelial-to-mesenchymal transition and promote growth. Additionally, degradation of the ECM by MMP activity removes physical constraints on cell growth. These effects are modeled by rule 1. It is also known that growth factor is depleted from the extracellular environment through receptor internalization by cells which bind growth factors. This effect has been observed in-vitro [4], and the process is represented within the model as the differential consumption of growth factor by proliferating cells. This process is controlled by rule 10.

## III. Model Behavior Perturbation and Comparison with Experimental Observations

In order to evaluate whether MMPs and TIMPs may be functioning as activators and inhibitors in morphogenesis, we compare results from published in-vivo experiments with output generated from corresponding changes to the simulation. Specifically we compare the effects of increased TIMP expression and inhibition of MMP activity on maximal duct length.

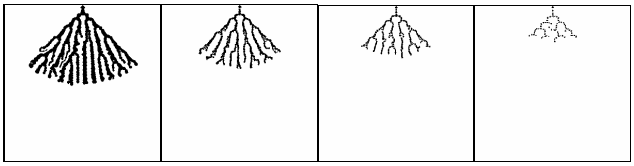
A. *Effect of changes in basal TIMP level*

Transgenic mice overexpressing human TIMP1 have been generated [2]. The TIMP1 transgene was driven by a β-actin promoter, resulting in constitutive expression. The effect of the introduction of TIMP1 is modeled through manipulation of the simulation parameter $h$. The results are summarized in Fig. 4.

B. *Effect of changes in MMP autocatalysis parameter*

There are 28 known MMP genes in humans. Broad-spectrum MMP inhibitors have been developed which are used to mimic the effect of knocking out all MMP genes. These inhibitors have been used to study the role of MMPs in branching morphogenesis. The effect of these inhibitors is imitated in the simulation by decreasing the value of the simulation parameter $c$. The results from this analysis are summarized in Fig. 5.

A.



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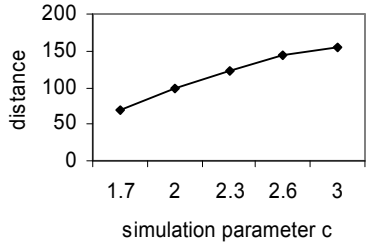


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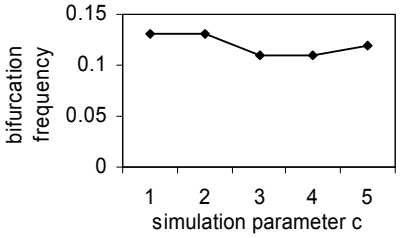


Fig. 5. Effect of reduction of *MMP* activation coefficient, *c*, on simulation output. A. Branching pattern after 300 steps, with $c = 3.0$, $c = 2.3$, $c = 2.0$, and $c = 1.7$, from left to right. B. Simulated duct extension distance for different values of *c*, after 300 steps, measured from the initiation point to the tip of the furthest simulated duct in points. C. The effect of *c* on the number of bifurcations per unit distance after 300 steps.

## V. Discussion

It has been shown that constitutive expression of TIMP1 in mice inhibited duct extension by approximately 25% compared to w.t. controls [2]. The simulated ducts behave in a similar fashion, with a significant reduction in simulated duct extension in response to increasing the basal level of the *TIMP* variable by increasing the parameter $h$ (Fig. 3A,B). The parameter $h$ also influences the number of simulated duct bifurcations events per unit distance (Fig. 3C). The effect of TIMP1 expression on the frequency of duct bifurcation events in-vivo has not been measured to our knowledge but such an analysis would be interesting in light of the simulation output.

The effect of broad-spectrum MMP inhibitors on mammary duct development in mice is quite dramatic, more so than the effect of TIMP-1 expression. The inhibitor GM6001 is able to completely block duct elongation during the initial 3 weeks of mamary gland development [2]. Although duct growth eventually occurs, the process is attenuated by continuous treatment, resulting in a ductal network that is approximately 50% of the size of untreated mice. Reduction in MMP activity is mimicked in the simulation by reducing the value of the parameter *c*. The simulated ducts respond in a fashion similar to ducts in-vivo, as decreasing values of *c* result in a slower rate of simulated duct extension (Fig. 4A, B). However, unlike the results from increasing the value of $h$ (Fig. 3C), there was not an apparent reduction in simulated duct bifurcations per unit distance (Fig. 4C). Again, it is not known whether a corresponding effect is observed in-vivo as a consequence of MMP inhibition although this could also be analyzed using existing data from in-vivo experiments.

## VI. Conclusion

The simulation model of mammary gland morphogenesis described in this report generates qualitative results that are similar to those observed in-vivo, in response to modulation of TIMP and MMP activity. The inhibition of simulated duct extension is consistent with the possibility that MMPs and TIMPs are serving as activators and inhibitors in a reaction-diffusion-like process. Additional quantitative data from in-vivo experiments is needed to test this hypothesis further. Comparison of simulation model behavior and experimental results will be useful in this effort.

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