Studies in Shape Homeostasis
Progress Report

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Abstract:

This report summarizes recent studies of the effects of microenvironment and metabolic network architecture on shape stability and reorganization in clusters of virtual cells. Knockdown mutations that confer a growth advantage on the mutant cell and its progeny induce a range of responses in self-organizing clusters (SOCs) of virtual cells, depending on which cell and in what state such mutations are introduced. These studies have implications for two main areas of biology and medicine: control of tissue size, and dysmorphogenesis associated with tumor formation.

Derivative models are described in a separate report, “Nutrient Uptake Models for Education”.

1. Introduction

Shape homeostasis in living tissues is an important but poorly understood phenomenon related to the general processes of allometric growth, tissue repair, regeneration, and cancer-related loss of normal controls on tissue size and morphology. Fundamentally the control of tissue organization involves at least three cellular processes –cell growth, division, and cell death, primarily apoptosis, for pruning unwanted or inappropriately placed cells. Even in the simplest multicellular organisms, shape homeostasis may also involve directed cell movements or differential adhesion and contraction, as documented in the classic sponge cell reorganization studies of Wilson (1908), formation of a migrating slug and fruiting body of slime molds, or, in more complex metazoa, morphogenetic movements during embryogenesis.

We have sought to understand the cellular basis of shape homeostasis through growth, division, and cell death controlled by local microenvironments, so called contingency, or responsiveness of cells to local conditions (Gerhart and Kirschner, 1997). Our general approach involves building virtual models of simple tissues that exhibit shape homeostasis to explore, at least in an abstract sense, candidate mechanisms for controlling tissue size and shape. These models do not involve directed cell movements.

2. Background

The models and experiments summarized below derive from a model of a “self-regulating cluster” of 21± cells in a compact shape, designed by Mason Vail. The model has been translated to run on v1.04, while retaining the primary emergent property of developing from a single virtual cell to form a stable compact shape containing ~21 cells (Figures 1-4). In addition, when a mutation conferring a proliferative advantage is introduced to a single cell in such a stable cluster, the cluster reorganizes and restabilizes at a larger size, dominated by the progeny of the mutant cell. The “knockdown” mutation reduces the sensitivity of cells to growth suppression by neighboring cells, and so creates an unbalanced state and subsequent competition between wildtype cells and mutant cells, as one might expect in early stages of tumor formation where normal cells compete with transformed cells for resources.

The goals of these studies are 1) to examine in a detailed and systematic way the range of responses of individual cells to such “knockdown” mutations and 2) to understand the mechanisms that favor stability or instability of such self-organizing clusters (SOCs).

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3. Results

3.1 Initial Studies

During May 2009, initial studies on the base model (translated to v1.04) were carried out. These simulations revealed that clusters could develop from a single cell and stabilize at 20-22 cells with compactness values between 0.5 and 0.6. Closer inspection of such a stable cluster revealed that its cells were in different states, for instance, near death, far from death, or in between (Figure 5). Cells near death ((pink – red, Fig. 5) were insensitive to a knockdown mutation that decreased the inhibitory effect of NeighborhoodMarker on Gene 2 (Division) from -1.34 to -0.8. However, the same mutation in other cells induced major reorganization of the cluster (Figures 2, 6, 7). Typically, reorganized clusters were more compact and larger than the initial stable form.

The above studies set the stage for a more detailed and systematic study of the sensitivity of particular cells to such knockdown mutations. In early June 2009 two summer interns, Drew Stritzke and Sasha Volgamore, joined CDR and after a brief period of introductory training and orientation, began to conduct experiments of SOCs, beginning with the model description SOC_Mod3. Modifications form the base model included substitution of fixed initial chemistry for Rigidity and Elasticity resources (instead of gene constructs), renaming “Floodgate” as “SecretoryFactors”, addition of a lineage marker (Gene 11), and setting Max steps = 8000, Stable steps = 500. These became the reference model description and conditions for the next series of experiments.

3.2 Factors affecting Cluster Stability

As studies progressed, it became clear that knockdown mutations could induce other events: many of the clusters became unstable after a knockdown mutation, engaged in endless cycling between phases of proliferation and cell death or fragmentation of the cluster into two or more groups of cells, one of which usually died. In addition, mutations could have minimal impact: 1 or 2 transient rounds of division, followed by death of any mutant cells. In such exceptional cases, the restabilized cluster contained the same number or more total cells than the cluster before mutation, including one or zero mutant cells, but in at least one case a 22-cell post-mutation stable form arose from a cluster of 23 cells.

We have begun to explore the factors that contribute to instability after a knockdown mutation. This can be quite challenging to isolate a particular factor or combination of factors that lead to instability, or appear to be necessary for achieving stability. One strategy is to reduce the complexity of the regulatory network by abstracting components or pathways that one surmises are incidental to the observed behaviors. For instance, one could reasonably presume that as long as adequate nutrient is available, the nutrient uptake pathway represented by Gene 0, EQ 0 and EQ 1 could be simplified in the model description as “constant initial chemistry” (Fig 4). Similarly, while the original baseline model represented elasticity and rigidity as gene products, substitution of constant values in initial chemistry is probably acceptable. These assumptions turn out to be dangerous and overly simplistic. For reasons not yet fully understood, variations of physical properties that accompany changes in cell size are probably important in achieving stability.

Much work remains to be done on this topic, but our provisional conclusions are as follows:

1. Rigidity and elasticity should be represented as gene products.
2. Cell adhesion strength may affect contact signaling, which protects cell from apoptosis. Changes in cell adhesion properties may impact cluster stability.
3. Because of (2) above, we expect that larger cell sizes and perhaps larger clusters of cells (more cells, larger cells, or both) may offer greater flexibility for modeling control of tissue size and organization.
4. Preliminary results show that nutrient levels may alter cluster behavior. Lowering [Nutrient] from 17.2 to 10.0 increases stability.
5. Regardless of the particular mechanisms involved, these simulations reveal that subtle differences in cell state affect the susceptibility of a cell to knockdown mutation, emphasizing the importance of cell context, not simply genetic alteration, in control of cell proliferation.

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4. Discussion

The studies described above are relevant to the broad goals of the TATRC tissue modeling research for at least two reasons. First, they demonstrate the effectiveness of using simulations and sets of experiments to unravel the details of network architecture and local environment that conspire to stabilize or destabilize small clusters of cells. Second, the successes of student interns show that in short order, individuals with no advanced training in biology or computer science can learn to use the client interface, run simulations, record them and analyze data, and alter model descriptions. The latter results show that, while the user interface is still being developed, its current form is useful and manageable.

In the coming weeks we intend to continue these experiments, consolidate our findings, and perhaps submit them for presentation at a research conference or include them in a larger work submitted for publication in a peer-reviewed journal.

5. Figures

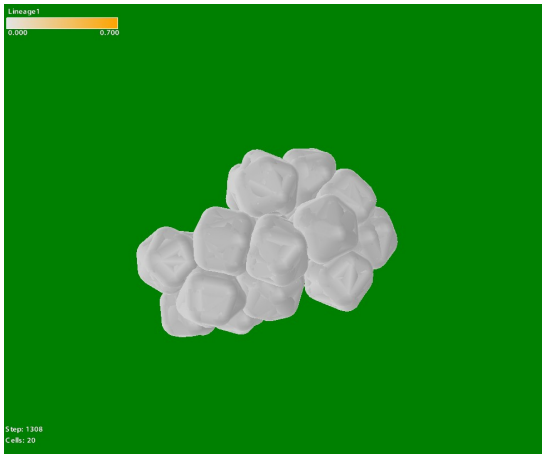

Figure 1: A stable 20-cell SOC.

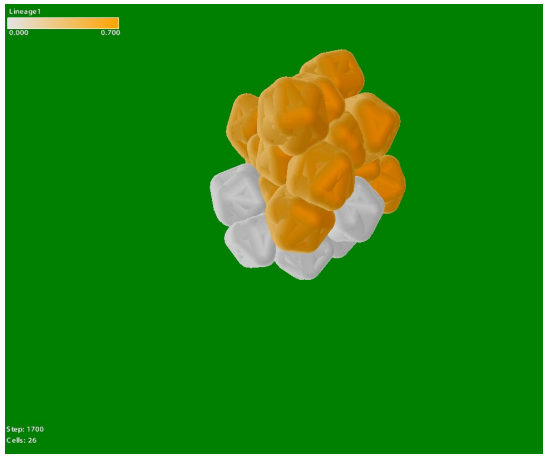

Figure 2: Mid-reorganization after a knockdown mutation (Orange cells = mutant progeny)

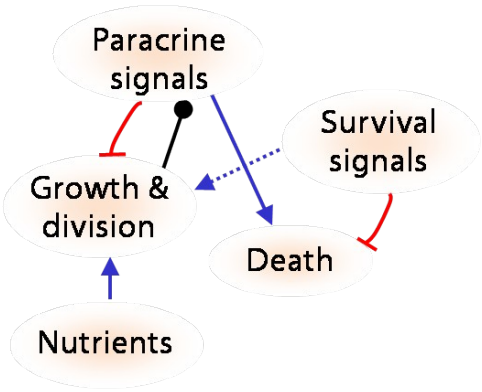

Figure 3: Abstracted mechanisms that regulate size & shape of SOCs. Paracrine signals limit cluster size and signals mediated by cell contact favor survival of interior cells and death of peripheral cells, thereby favoring compactness.

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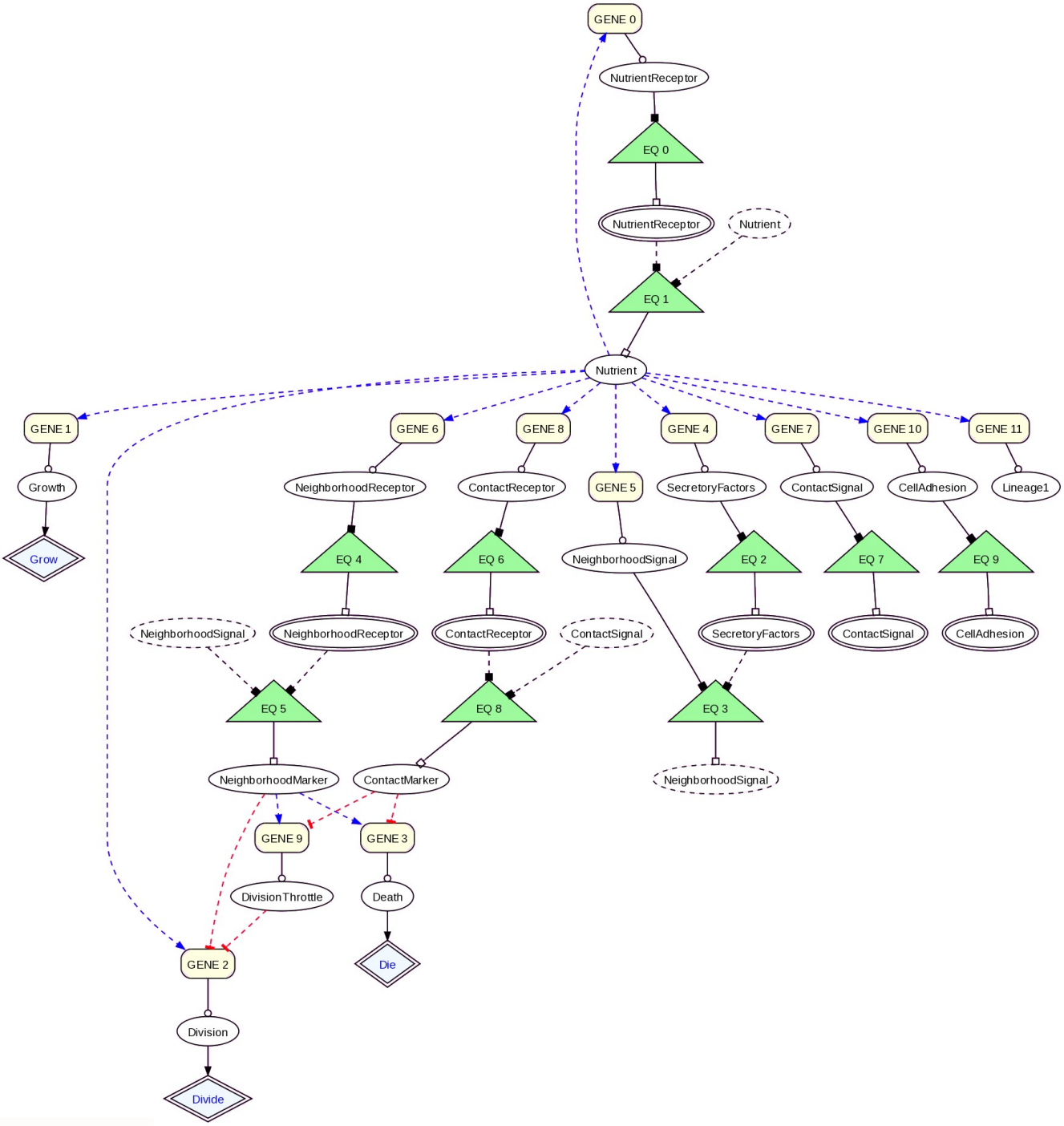

Figure 4: Metabolic network of an SOC, elaborated from the abstract mechanisms shown in Figure 3.

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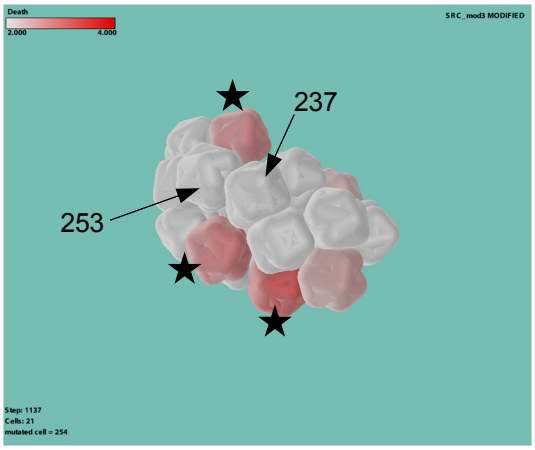

Figure 5: A 21-cell SOC has cells in different states. Knockdown mutation has little or no effect on cells near death (stars).

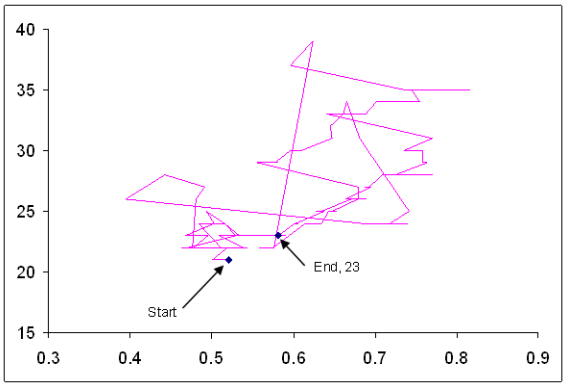

Figure 6: Reorganization after KD mutation in cell 237 (see Fig. 5). The cluster begins as 21 cells and restabilizes in a more compact 23-cell form containing only 1 wild type cell..

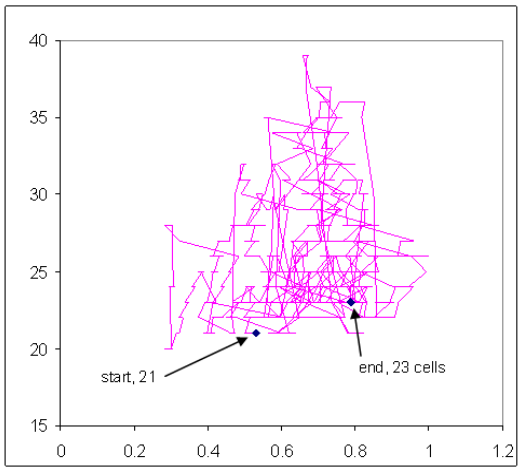

Figure 7: Reorganization after KD mutation in cell 253 (see Fig. 5). After protracted bouts of division, growth and death, the cluster restabilizes in a more compact form containing 23 mutant cells.

6. References

Gerhart, J and M Kirschner (1997) *Cells, Embryos, and Evolution*. Blackwell Science, MA.

Wilson, HV (1908) On some phenomena of coalescence and regeneration in sponges. J Exp Zool 5: 245-258.

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