Evolutionary Systems Biology integration of multi-level CTMC interaction models of biochemistry and cancer cell growth using Evolvix.

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Abstract

While biochemistry evidently affects the growth rate of cells, many biochemists routinely ignore population variation, just like population geneticists usually ignore causal details of biochemistry that underpin a change in growth rate caused by a mutation. A true EvoSysBio integration requires an explicit mechanism for how molecular reaction rates affect the reproduction rates that determine the fitness of an organism. Here we simulate a very simple and completely explicit Continuous-Time Markov Chain (CTMC) model of cancer cells whose growth rate is affected by the biochemical equilibrium between two molecular complexes. Approximately 70% of breast cancers are of a type that overexpress Estrogen Receptor-alpha (ERα). Cell growth in this type of cancer is inhibited by hormonal therapies that antagonize ERα function as a transcription factor. ERα is encoded by the ESR1 gene, which itself is a target of ERα mediated transcription. When activated by estrogen, ERα binds to the ESR1 promoter, repressing new synthesis of ERα protein. Estrogen binding also induces pathways that lead to degradation of ERα protein. This negative feedback loop is finely tuned to natural levels of estrogen and results in natural levels of growth. In breast cancer, the system is thrown off its natural course such that increased levels of ERα induce levels of cell growth that can lead to cancer. Thus, both genetic changes to the ESR1 promoter, ERα protein degradation, and biochemical changes in estrogen metabolism can effectively cause changes in cell growth rates, which can be seen as the 'fitness' of a cancer cell. Predicting cancer cell growth in this system raises a conceptual multi-level simulation problem, because the molecular aspects of this model need to compute the biochemistry in a way that influences growth rates at the cellular level, without resetting growth at each cell division. We present progress towards addressing this simulation challenge in pure mass-action models, which we implemented using the Evolvix model description language. We found that such models can be constructed in more than one way. We explored some candidate model properties that could aid efforts to develop abstractions for more efficiently simulating the common multi-level modeling problems behind many important biological questions. These efforts are ongoing and aim to find efficient ways of encoding and exploring such models *in silico*. In particular, we are investigating how architecting a new compiler for a general-purpose programming language for biology could improve the efficiency of analyzing the dynamic multi-level simulation scenarios that characterize many questions in EvoSysBio. Progress can be followed at http://evolvix.org.

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An earlier version of this abstract was presented as a poster at the 2017 Symposium on Evolutionary Systems Biology of Cells held at the International Conference of the Society for Molecular Biology and Evolution (SMBE), 2017 July, 2-6th, Austin, Texas (see http://www.smbe2017.org/). Updates to this model will follow eventually.

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Introduction

Nested replicators are central to biology: cells replicate in organisms, molecules replicate in cells. Each replicator is dynamically changing. This complicates predictions of what will happen next in biological systems.

Mathematical models' importance for the study of dynamic, multi-level, biological systems has grown, but models currently rarely span more than one level.

Biochemistry affects growth rates of cells yet many biochemical models ignore population consequences. In turn, most population models ignore biochemical details that affect growth and are changed by mutations.[5] EvoSysBio fitness landscapes require full integration.[6-8]

Cancer Background

About 70% of breast cancers are ERα-positive, hinting at overexpression of Estrogen Receptor-alpha. Cell growth in this type of cancer is inhibited by hormonal therapies that antagonize ERα's function as a transcription factor. ERα is encoded by the ESR1 gene, which itself is a target of ERα-mediated transcription. When activated by estrogen, ERα binds to the ESR1 promoter, repressing new synthesis of ERα protein. Estrogen binding also induces pathways that lead to degradation of ERα. This negative feedback loop is finely tuned to natural levels of estrogen and results in natural levels of growth. **In breast cancer, the system is thrown off its natural course such that increased levels of ERα induce levels of cell growth that lead to cancer.** Thus, genetic changes to the ESR1 promoter, ERα protein degradation, and biochemical changes in estrogen metabolism can effectively change the rate of cell growth, which can be seen as the 'fitness' of a cancer cell.

Modeling

Our basic models track the relative molecular abundance of the ERα transcription factor. When overabundant, ERα can stimulate cell growth and cells become cancerous[1].

We use the Evolvix modeling language[9] (Free Prototype 0.3.1). **It allows us to efficiently explore diverse models and focus on the biology instead of model construction.** It simulates pure mass-action systems observing amounts of interacting parts. It uses known methods[9-12] to simulate Continuous-Time Markov Chains using various methods.

Main Question

How can we simulate dynamic, multi-level systems *without losing or resetting* relevant lower-level biochemical states when upper-level actions occur at the cell level, and thus change, duplicate, or destroy the very container in which the lower-level states have been defined?

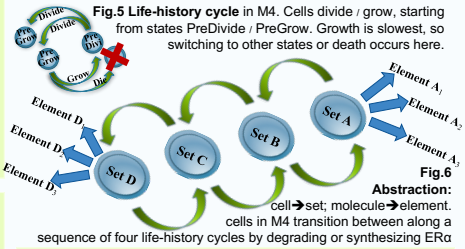


Fig.5 Life-history cycle in M4. Cells divide / grow, starting from states PreDivide / PreGrow. Growth is slowest, so switching to other states or death occurs here.

Fig.6 Abstraction: cell➔set; molecule➔element. cells in M4 transition between along a sequence of four life-history cycles by degrading or synthesizing ERα

The Nesting Problem

Why is it so difficult to consider biochemical changes in the context of replicating cells?

Let's consider the count of ERα in a simplified cancer cell model in which nothing else mattered. We can then distinguish different cells as being in different states if they contain different counts of ERα. Since this could be thousands of molecules, our small nested example already has thousands of different "types of cells". However, these "cell types" only differ by one molecule more or less from their neighboring types. This suggests that very similar states could be lumped together if their behavior is indeed identical. If true, we can reduce the problem of 'too many types of cells' by combining cells with similar phenotypes into 'collective types'.

Remaining problem: the dynamics of molecules inside of cells is ultimately determined by their biochemistry, which is not necessarily reset by cell division. **This can lead to many complicated dependencies that may last for generations.** Thus, two daughter cells might be in the same biochemical state as their ancestor cell, just by physical continuity. Such states remain stable, *until* the relevant biochemistry is changed again. Thus, we have a complex multi-level system, in which the growth of cell types depends on *their* respective biochemical state *inside*. The same principle allows for transmitting information on DNA; only that DNA is stabilized in many ways, while most biochemical states are not.

Results

Here we investigate a simplified breast cancer cell model informed by observed data[1], which indicates that the growth rate of such a cell is affected by its molecular state. We use this model as a basis for investigating our main question from different angles.

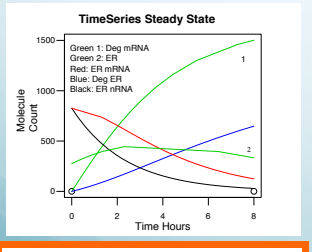
Chance or necessity? System dynamics are stochastic or deterministic, as governed by how many parts are available for each relevant action. In deterministic models *even tiny fractions of a part* can interact as if thousands existed; if the latter is true *for each important part*, such models work well. In contrast, stochastic models insist that *actions only occur if all required parts exist as requested,* leading to random waiting times and chance events. **We bio-curated**[13] the amount of average ERα mRNA per cell and found about 800 molecules per cell[2,3,4]. The cutoff for ERα-negative cells becoming ERα-positive[3] was about 80. These intermediate amounts justify analyzing our model using deterministic *and* stochastic simulations.

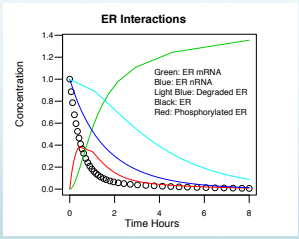
Modeling Conclusions

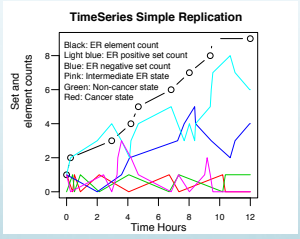
M1 & M2 time series match published simulations[1] fitted to observed relative molecular amounts (Fig.1-2). **M3** cells quickly switch between 2 biochemical states with longer / shorter waits for cell division. Passing through a rare brief transient state (Fig.3, pink) shared by new cells is enough to force mixing of both life history cycles; unlikely for mitotic cancer cells, maybe relevant for strong anisogamy models. **M4** has 4 complete life-history cycles for all 4 ERα counts allowed in its Lo / Hi growth cells (Fig.5). These could be independent, but are linked here in ERα incr-/decreasing transitions (Fig.6). Cell and ERα counts change separately.

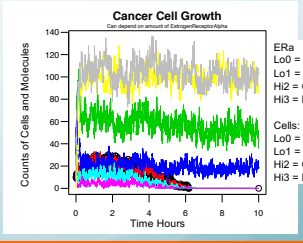
Discussion

ERα is a key regulator in normal physiology, as well as disease development. To improve our understanding of the ERα regulation system, we need to model more of what ERα does in the cell. This includes dimerization, binding to estrogen response elements in target genes, and activating or repressing gene expression. The impact of ERα on cell growth requires better multi-level models for understanding cancer. How to simulate these models is an active area of research[14-17], and of crucial importance for the EvoSysBio goal of constructing fitness landscapes[6-8].









Model M1: Steady State[1]

Basic 1-level model of molecular interactions representing steady state of ERα-positive breast cancer cell line MCF-7. Determines level of ERα, which is an important switch for cell growth. Cell division not modeled. No treatment.

Model M2: ERα after treatment[1]

Basic 1-level model of molecular interactions representing cell states after treating the cell line with estrogen. Shows interactions of the ERα regulatory system. Estrogen treatment degrades ERα to non cancer levels. No cell division.

Model M3: Simplistic Replication

Simplest 2-level model. Two biochemical states affect cell division rate, but after replication, the new cell resets into a shared transient state. No reset of parent cell. Partial resetting means: More work to do, as our main question remains.

Model M4: Over-Separated

Separate for these 4 cell types (Lo0 Lo1 Hi2 Hi3):

- Set **Counts** of *PreGrowCells* and *PreDiviCells*
- ERα Element **Counts** in all *PreGrow*, *PreDivi*
- Loss of ERα cell locations, but cell type is kept
- Change Set *and* El counts at LifeCycleEvents

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