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# Models of cell signaling pathways

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Cellular signaling circuits handle an enormous range of computations. Beyond the housekeeping, replicating and other functions of individual cells, signaling circuits must implement the immensely complex logic of development and function of multicellular organisms. Computer models are useful tools to understand this complexity. Recent studies have extended such models to include electrical, mechanical and spatial details of signaling, and to address the stochastic effects that arise when small numbers of molecules interact. Increasing numbers of models have been developed in close conjunction with experiments, and this interplay gives a deeper and more reliable insight into signaling function.

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## Abbreviations

**EGFR** epidermal growth factor receptor  
**MAPK** mitogen activated protein kinase

## Introduction

Modeling biology, especially development, is a hazardous undertaking [1]. It is an enormous task to assemble diverse kinds of biological data into a form that a computer can digest. At the end of this process, it is natural to ask, what is the model worth? Unfortunately, biological model predictability is specific, and highly dependent on the parameters applicable to a given system. Evolution has layered so much complexity over the underlying physical laws that models are apt to run aground on simplifying assumptions. The availability of outrageous amounts of high-throughput biological data finally endorses the reductionist approach for tackling biological questions [2,3]. In this review, I focus on intracellular signaling rather than communication between cells. I first introduce different kinds of signaling models and consider what current modeling tools can do. I then turn to some specific signaling phenomena that are particularly

interesting for development: growth factor pathways, cellular rhythms, and cytoskeletal mechanics. I neglect many additional modeling efforts in this brief review, which is only able to give a glimpse of the diversity of cellular signaling processes now amenable to modeling.

## Modeling

### Scope

Cell signaling is, loosely, the interface between genes and everything else in the cell. Often the term is restricted to the biochemical events that convey cellular information. The vast majority of current signaling models fall into this category. However, the correspondence between biology and the model should, in principle, improve as models take more biological features into account. A broader interpretation of signaling could include almost any form of information flow, including many cell biological and structural events that shape signaling (Figure 1). A central issue in all forms of modeling is that of how much biological detail to include. This detail could be in the form of complexity of the pathway circuit, or in terms of greater precision in specifying what happens within each pathway.

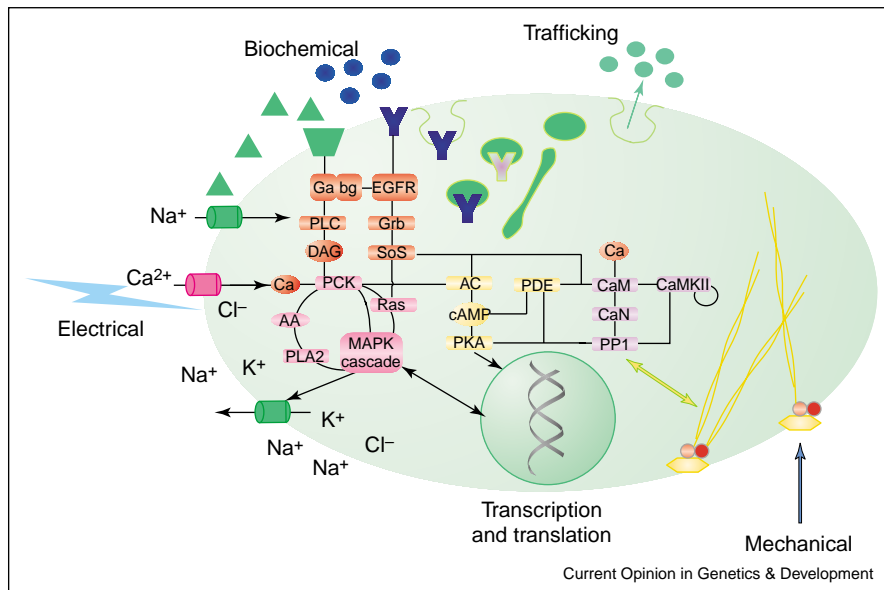
### Complexity

In cell signaling and developmental models it is particularly difficult to decide how many pathways to include in the model. The major receptors and outputs may be known, but crosstalk and regulatory inputs are often critical in determining pathway function. At a qualitative level, there are attempts to represent this complexity by generating comprehensive signaling pathway diagrams [4] and molecule interaction databases [5]. However, predictive models of signaling need more detail than just connectivity information. A reaction-level signaling model, for example, requires system-specific parameters such as rate constants in addition to the general reaction diagram. Interestingly, current quantitative modeling efforts appear to have hit a ceiling of ~200 molecular species (e.g. [6]). This apparent barrier indicates that another level of interface may be needed to scale modeling efforts further.

### Model detail

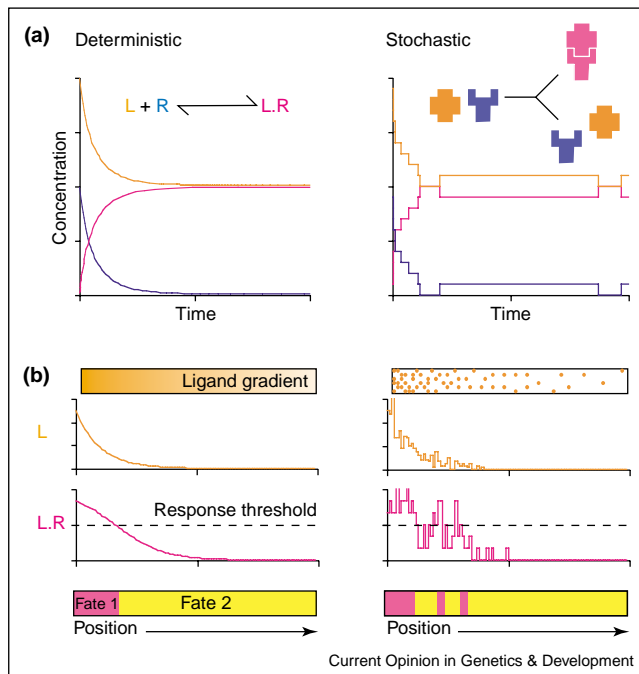
The other challenge, limited by experiments as well as by programming ingenuity and computer power, is the handling of ever-finer levels of molecular detail. Consider the simulation of ligand–receptor interactions (Figure 2). At the simplest level, this is simply an exercise in mass-action kinetics, and the response is smooth and deterministic (Figure 2a). If we now consider that ligands and receptors may be present in only a few tens of copies on a

Figure 1



Interrelated forms of cellular signaling. Biochemical signaling involves a complex network of pathways that link together different functions of the cell. Electrical inputs contribute via calcium influx to the chemical network, and the ion channels are, in turn, modulated by phosphorylation and turnover. Trafficking controls receptor availability and the compartmentalization of many signaling events, and is, in turn, biochemically regulated. Mechanical signals lead to formation of biochemical and mechanical signaling complexes. The whole network is tightly coupled to transcriptional and translational events.

Figure 2



Representing ligand–receptor coupling at mass-action (deterministic) and individual-molecule (stochastic) levels of detail. L represents ligand, R represents receptor, and L.R is the ligand–receptor complex. (a) Time-course of ligand–receptor binding reaction where there is a limiting amount of receptor. In the deterministic situation, the

given cell, then the reaction should be treated as the probabilistic (stochastic) collision of individual molecules. The response is noisy and its outcome is no longer deterministic.

In the real world, the position of ligand and receptor matters. Here we again can choose to model effects either deterministically or stochastically (Figure 2b). Biochemical fluctuations due to stochasticity introduce issues of noise tolerance in signaling (see review by Kerszberg, this issue). Several studies (e.g. [7]) have analyzed diffusive effects on ligand–receptor interaction, and indeed, the formation of ligand/receptor gradients is an essential

amount of bound receptor (L.R) smoothly increases and settles to a final state where most of the receptor is complexed with the ligand. In many cellular reactions, very small numbers of molecules interact and it is more correct to represent this using probabilistic (stochastic) events for the binding reactions. In this situation, the individual binding and unbinding reactions occur in a chance manner. These fluctuations continue even after the deterministic system reaches equilibrium and are a source of molecular noise. (b) Ligand–receptor binding in a developmental situation where the ligand is present in a gradient. The deterministic calculations give a smooth decrease of ligand–receptor (L.R) complex with distance from the ligand source. If there are small numbers of molecules then there is again noise as a result of stochasticity. If the developmental program uses a threshold of L.R levels to determine cell fate, then the stochastic case can lead to noisy decisions when very small numbers of molecules are involved. Biochemical noise is therefore an important aspect of cellular signaling and may need to be modeled explicitly.

component of many theories of morphogenesis [8] (see review by Eldar, Shilo and Barkai, this issue).

At an even finer level, the physical organization of the receptors on the surface of the cell may be important in determining signal propagation [9] and assembly of signaling complexes [10]. There are still further levels of molecular detail relevant to cellular signaling, including attempts to utilize Brownian dynamics and protein structure prediction to estimate key interaction parameters [11]. These are largely unsolved problems, and it seems a very long way from protein structure to analyzing signaling networks.

In principle, a similar series of ever-finer levels of analysis could be followed with other aspects of cellular signaling. For example, the mechanical outcomes of signaling are critical in development [12]. There are increasingly sophisticated experiments and simulation tools to analyze the details of how cells deform and move [13].

### Signaling pathway models and developmental mechanisms

Development involves many forms of biological interactions. Many completely different kinds of models have been proposed for each of these domains, including the mechanical interactions of gastrulation [14], the formation of morphogen gradients [8], and the genetic circuits that specify the developmental program [15]. Signaling pathways are the glue that link all these phenomena together, and the models are correspondingly diverse. There are some clusters of modeling efforts on pathways in systems where there is a fortuitous combination of data availability and biological significance. However, there are many models that defy categorization. On the basis of their relevance to development, I focus here on three broad areas of signaling models: growth factor pathways, cellular

rhythms, and on the emerging area of cytoskeletal interactions with signaling.

### Growth factor pathways

Growth factors are particularly interesting both from the biological viewpoint and in terms of the diversity of computational roles they play. Many growth factors feed into the mitogen activated protein kinase (MAPK) cascade and its variants. One of the long-standing issues about MAPK signaling has been how a single pathway can give rise to distinct kinds of response. These include the functions of switching, oscillation, high amplification, and thresholding. From a systems biology viewpoint, all are interesting and related, and from a developmental viewpoint each may be a useful module. With the appropriate assumptions, the MAPK cascade can be persuaded to do all these things (Table 1).

Differential receptor trafficking and subsequent compartmentalization has been analyzed as an upstream mechanism for diverse responses [16,17,18]. A range of responses can also be elicited from the kinase cascade depending on inhibitory phosphatase feedback via MAPK phosphatase transcriptional regulation [19] and on expression of kinases and phosphatases [20,21]. It is worth noting that several of these studies combine simulations and experiments, so the versatility of the pathway is not entirely a figment of the modeler's imagination.

The flip side of versatility is robustness under a range of conditions, which is important for reliable development (see review by Kerszberg, this issue). The architecture of the pathway contributes to this with redundant activation mechanisms [22]. Additionally, the kinetic rates of key pathway steps have been shown by a combination of simulations and experiments to lead to stable behavior

**Table 1**

#### Multiple properties of the MAPK pathway.

Function	Possible mechanisms
Amplification	- Multiple enzymatic amplification stages: at the receptor, then dual phosphorylation steps for MAPK kinase and MAPK. - Positive feedback from MAPK back to its upstream activators.
Alternative responses	- Selective receptor/adaptor trafficking depending on different ligand-receptor binding properties. - Stimulus-duration-dependent switching into active state.
Ultrasensitivity/thresholding	- Positive feedback. - High-order multiple amplification stages.
Sustained activation	- Positive feedback loop. Activated MAPK increases activity of upstream enzymes, and under restricted conditions this can be self-sustaining.
Transient activation	- Receptor internalization. - Negative feedback onto adaptor proteins from receptor. - Negative feedback via transcriptional activation of inhibitory phosphatase.
Oscillation	- Negative feedback with phase delay.
Robustness in response	- Redundant activation mechanisms. - Saturation of internal enzyme steps.

over a wide stimulus range [23]. A similar combined approach has addressed the temperature dependence of activation of different downstream pathways of MAPK [24\*].

Recent simulations based on high-resolution imaging methods have brought spatial considerations to the analysis of MAPK signaling [17\*\*,25]. Spatial effects across multiple cells have clear implications for developmental patterning. One of the interesting findings is that the EGFR/MAPK system can lead to signal propagation either at the level of the receptor [26\*\*] or at the level of multiple cells in autocrine relays [27].

The control of nuclear cycling is an important end-point of several growth factor pathways. Studies combining experiments and simulations show that this cycling may be a means for calibrating expression to receptor activation levels through the Janus family of kinases [28,29].

### **Cellular rhythms**

A fruitful area of model–experiment collaboration has been in the study of cellular rhythms [30]. The cell cycle is perhaps the most important of these periodic events from the genetics and development viewpoint. Although many of the key players of the cell-cycle network are known, the specific rate constants have been difficult to obtain. Therefore several studies have studied the behavior of cell-cycle networks of known architecture using techniques that explore wide ranges of parameters and identify ranges in which interesting effects occur. There are several stable states that mark different stages of the cell cycle, and the transitions between these states have been shown to occur as the cell-cycle control signals build up ([31–33,34\*]; reviewed in [35]). A common node between the processes of cell cycling and apoptosis suggests mechanisms for interaction between these phenomena [36]. At a more detailed level, it has been possible to relate the kinetics of DNA replication to experimental data [37\*].

The circadian rhythm is another major cellular oscillator that has long been a subject for modeling ([38,39]; reviewed in [30]). A central aspect of such oscillators is their entrainment to the light–dark cycle, and multiple feedback loops may contribute to the robustness of this entrainment [40,41]. As another manifestation of robustness, this phase-locking may persist even in the presence of stochastic noise [42–44]. Entrainment may also occur with respect to temperature, especially in plants [45,46]. The clock is a very complex system, involving transcriptional/translational feedback and influencing cellular functions from development to metabolism. Evolutionary arguments are valuable in considering how the effect of the clock is propagated to control these different cellular functions [47–49].

The somitogenesis clock is an interesting combination of timing and spatial effects that has been suggested to form the basis for vertebrate segmentation (reviewed in [50] and by Giudicelli and Lewis in this issue). Here a periodic clock is present in a series of synchronously oscillating cells. The state of the clock is frozen in response to a wave of cell determination propagating along the cells. Differentiation then proceeds according to the state specified by clock signals, giving rise to periodic patterns in space.

### **Mechanics**

Mechanical aspects of morphogenesis represent a major subfield of simulations in development [14]. Here, I only consider intracellular mechanical simulations, especially with a view to understanding how the intricate cytoskeletal architecture may self-organize in the context of cellular signals and forces. Cell division is one example of dynamic assembly and breakdown of mechanical structures. The mitotic spindle self-organizes, is resistant to many mechanical perturbations, and is dependent on motor function. The formation of microtubule asters can be described by the stochastic binding and movement of motor complexes that can bind to and traverse pairs of microtubules, one from each aster [51]. The feedback between cellular stresses and externally applied forces can lead to alignment of the mitotic plane that, in turn, influences the shape of developing epithelium [52]. A similar feedback has been analyzed for neutrophils in a detailed mechanical model that addresses several experimental situations [53]. An extremely detailed stochastic simulation of the formation of the intermediate filament network in the epithelial cell following mitosis shows how stress-induced local synthesis of proteins can lead to appropriate formation of the cytokeratin network [54\*\*]. Stochasticity may also play a key role in the specifics of cell polarization. Actin polymerization through local stochastic activation of Cdc42 can lead to formation of cell polarity without any other physical cues [55\*].

### **Testing model predictions**

A critical step in model development is to go from explaining observations to making predictions. I conclude with two examples of predictive roles for modeling: bistability in signaling, and synthetic biological oscillators. One of the interesting hypotheses regarding the MAPK cascade as well as other signaling systems is that they might function in the manner of a bistable switch. Similar to a light switch, bistable systems have two stable states: one of low activity and one of high activity. A common way to achieve bistability in a signaling circuit is to have positive feedback. Here the output of the cascade connects either directly or indirectly back to the input, to activate itself. At least two models have proposed this kind of bistability in the MAPK cascade [6,56]. It has also been postulated for EGFR activation [26\*\*] and for several steps of the cell cycle [35]. There are several

predictions from such models which have influenced the design of subsequent experiments, and have gained experimental support. These predictions include the following. First, the response of the pathway should have a very sharp threshold with respect to a stimulus such as EGF [19,26,56] and should exhibit hysteresis [34]. Second, the system should generate a sustained response to brief stimuli [19,56]. Third, the blockage of any of the steps of the feedback loop should abolish the first and second properties [19,26]. Fourth, a measurement of local activity across a number of samples with local bistable loops should be bimodal, as some samples would be in the low-activity state, and others in the high-activity state [26,56]. A more impressive prediction from the bistability hypothesis comes from model behavior in space. If a sheet of tissue exhibits local bistability, and if some molecules in the bistable circuit can diffuse, then activity in one region should be able to propagate through the sheet. This occurs because activated molecules from the first region diffuse to their surround, and switch the neighboring regions to the active state. This propagating behaviour has also been observed [26].

A particularly clear illustration of the power of modeling in understanding signaling circuits comes through the design of oscillatory circuits based on models, and then testing these designs in engineered organisms [57,58]. Although these studies are not related to development directly, the ability to design desired circuit functions systematically is a good indication that modeling has abstracted at least some of the fundamental principles.

## Conclusions

An encouraging shift has taken place in signaling models as they evolve from their initial phase of novelty and speculative design into a powerful tool generally available to biologists. One of the striking trends of work in recent years has been the close coupling between model and experiment, as evidenced by several interdisciplinary studies. With the closer ties to experiment have come more stringent demands on biological realism, and another clear direction is the expansion from simple chemical models to include space, stochasticity, and even mechanical effects. Models are very demanding of experimental input for quantitative data, and the recent flood of high-throughput data and high-resolution imaging should return the favor, to the benefit of the field as a whole.

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