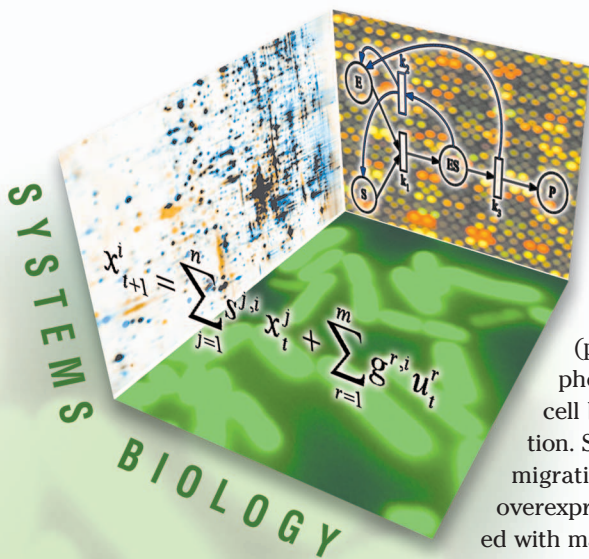


By Stanislav Y. Shvartsman, H. Steven Wiley,  
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# Epidermal Growth Factor Receptor Signaling in Tissues

Spatiotemporal dynamics of autocrine loops  
in the epidermal growth factor receptor system



In 1962, a peptide purified from the salivary gland of a mouse was shown to accelerate incisor eruption and eyelid opening in newborn mice [1]. The peptide was named epidermal growth factor (EGF), and soon afterwards, EGF and members of this family of peptide growth factors had been identified in numerous physiological and pathological contexts. EGF binds to a cell surface EGF receptor (EGFR), which induces a biochemical modification (phosphorylation) of the receptor's cytoplasmic tail (see Figure 1). The phosphorylated receptor then activates biochemical pathways within the cell by acting as an assembly site for enzymes that mediate signal transduction. Signaling regulates processes that affect cell division, differentiation, and migration [2], [3]. Abnormal EGFR signaling, due to overactive receptors or overexpressed ligands, can lead to developmental defects and has been associated with many types of cancers [4]. This much was understood about the EGF system when the 1986 Nobel Prize in Medicine was awarded to Rita Levi-Montalcini and Stanley Cohen for the discovery of EGF and nerve growth factor (NGF).

Today, the EGFR is the subject of more than 30,000 research papers. Many individual molecules mediating the EGFR-induced responses have been identified and are targets of development in oncology and other areas of medicine. Genomics and proteomics approaches will soon make it possible to follow all genes and protein/protein interactions affected by EGFR/ligand binding on the cell surface [5], [6]. The structural details of the interaction between EGFR and its ligands are becoming better understood at the atomic level and can be followed in real time with modern imaging tools. Despite these advances, neither the contribution of EGFR to tissue morphogenesis in development nor the exact role of deregulated EGFR signaling in disease processes is understood at this time [3].

There is a growing consensus in the research community that, in addition to cellular and molecular studies, the dynamics of the EGFR network and its operation must be examined in tissues. A key challenge is to integrate the existing molecular and cellular

## Glossary

**Autocrine:** Form of signaling in which a secreted ligand is recaptured by receptors on the same cell. See paracrine.

**Epithelium:** Epithelium is one of the primary tissues. All epithelial tissues are found on a surface. They are distinguished from each other by their differences in cell shape and cell orientation. In all cases the cells are tightly connected.

**Endocytosis:** A process in which a substance gains entry into a cell without passing through the cell membrane.

**Gene Expression:** All processes by which a mature protein is produced from the gene that encodes it.

**Growth Factor:** Proteins that regulate many cellular processes, including cell division. Examples include EGF (epidermal growth factor) and the NGF (nerve growth factor).

**Homeostasis:** The tendency of a cell or aggregate of cells to maintain a constant internal state despite a wide range of external disturbances.

**In Vivo:** Literal translation: in life. Refers to experiments performed in a whole organism rather than in cell culture. See in vitro.

**In Vitro:** Literal translation: in glass. Refers to experiments performed in cell culture as opposed to the cell's natural environment, such as the whole organism.

**Ligand:** A molecule, such as a growth factor, that binds to and activates a cell surface receptor. See receptor.

**Mitogen Activated Protein Kinase (MAPK) Cascade:** An intracellular signal transduction pathway that can be stimulated by multiple extracellular signals, such as peptide growth factors. This pathway is evolutionarily conserved, consisting of three protein kinases. Once activated, the final protein in this pathway, MAPK, is translocated to the nucleus to affect gene expression. See signal transduction.

**Paracrine:** Form of signaling in which the signal is received by cells near the transmitting cell.

**Receptor:** A molecule, usually membrane bound, that binds a ligand. Whereas the ligand represents the signal, the receptor represents the receiver of the signal and the transmitter of the signal into the cell.

**Signal Transduction:** The process by which an extracellular signal is transmitted into the cell, typically through ligand-receptor interactions. Often, the signal's ultimate destination is the nucleus, altering the cell's gene expression. An example of an intracellular pathway that mediates signal transduction is the MAPK cascade.

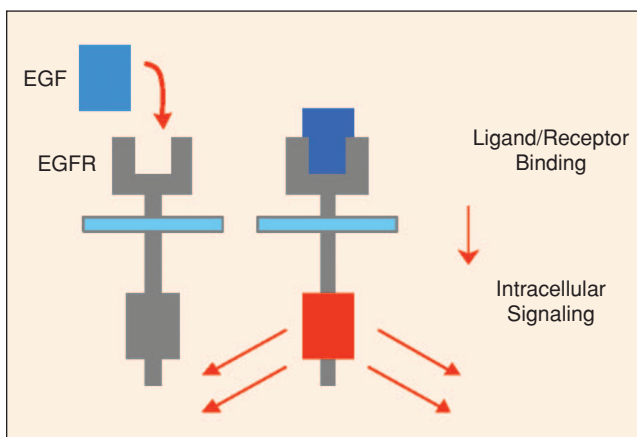
information into a systems-level description of the EGFR network at the tissue and organism level. The development of a systems-level description of EGFR biology in tissues is the main goal of much of the research in our laboratories. Here we describe two examples of EGFR signaling in tissues

and summarize our recent efforts in modeling EGFR autocrine loops, which is a predominant mode of EGFR activation in vivo.

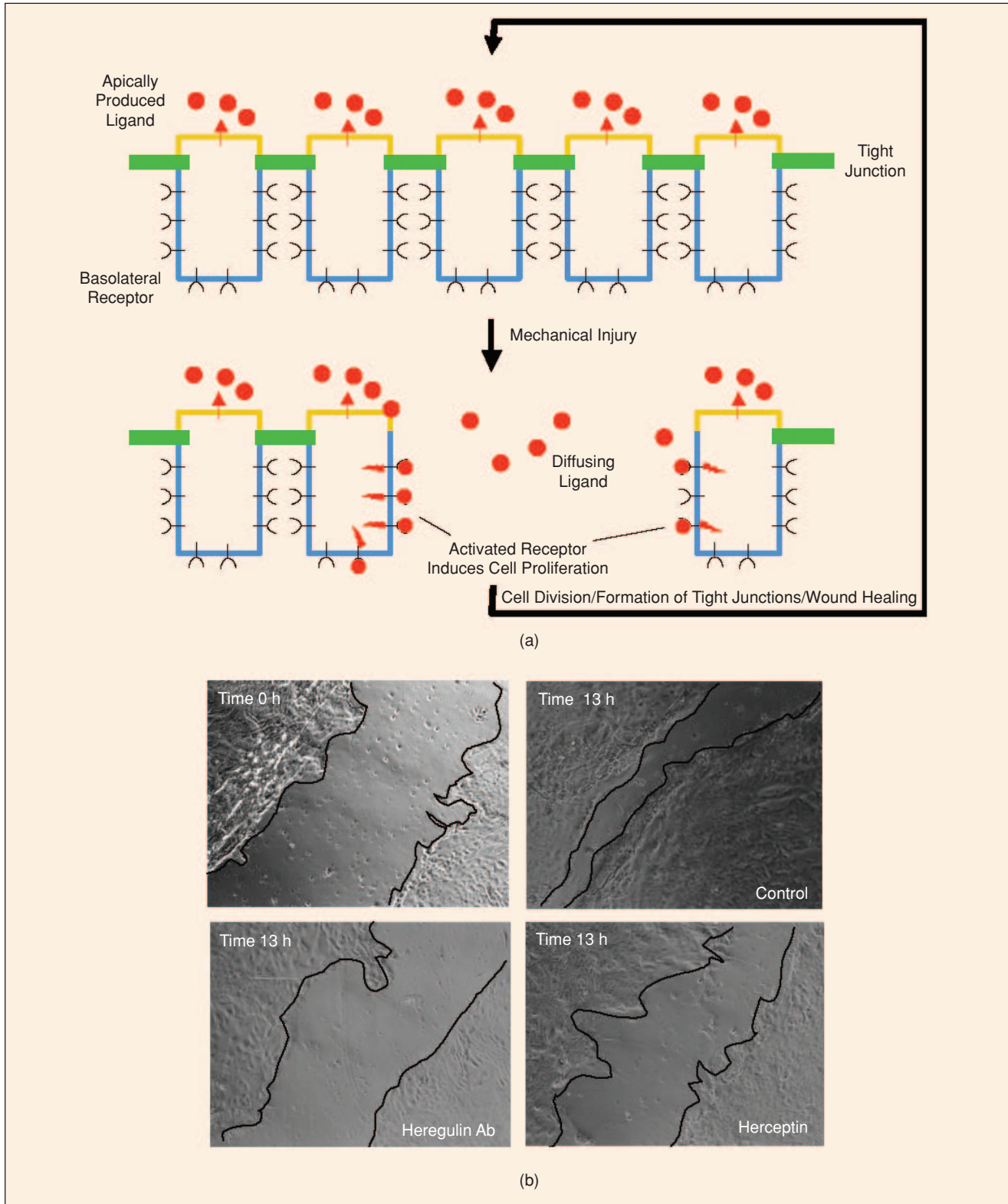
## Two Examples of EGFR-Mediated Responses in Tissues

EGFR activation in vivo is tightly controlled, both in time and in space. According to the currently accepted paradigm, receptor activation in vivo is controlled by ligand availability. This means that the receptor is present in excess and is waiting to be activated by locally produced ligands. Commonly, the cells expressing the receptor also express the activating ligand. To prevent erroneous receptor activation, the ligand and receptor must be kept physically separated. Alternatively, ligand production can be limited by the availability or activity of ligand-releasing enzymes. In this section, we describe how both strategies are employed in embryonic development and tissue homeostasis.

Physical separation of a member of the EGFR family and its ligands has recently been identified as the basis of an elegant mechanism for airway epithelium repair [7]. The membranes of epithelial cells that line the airway tract are compartmentalized into apical and basolateral compartments by tight junctions that comprise the physical



**Figure 1.** Mechanism of EGF receptor activation. EGF-like peptides induce receptor activation and cellular responses by binding to the cell surface receptor and activating its catalytic activity.



**Figure 2.** Role of autocrine signaling in wound repair. (a) A mechanism for repairing the epithelial layer relies on an autocrine loop, a mode of cell signaling whereby the cell simultaneously expresses the ligand and the receptor that can bind this ligand (see text for details). (b) The autocrine mechanism can be verified by experiments with receptor blockade or ligand removal by soluble antibodies. The phase-contrast microscopy images show the injured epithelial layers; the width of the wound at the start of the experiment is shown at time 0 h. At 13 h postinjury, the width of the control wound decreases while that of the layers treated with neutralizing antiligand or antireceptor antibodies remains unchanged.

attachments between individual cells. Each cell synthesizes both heregulin, a member of the EGF-like family of growth factors, and its cognate receptor. The ligand is secreted from the

## There is a growing consensus in the research community that the dynamics of the EGFR network and its operation must be examined in tissues.

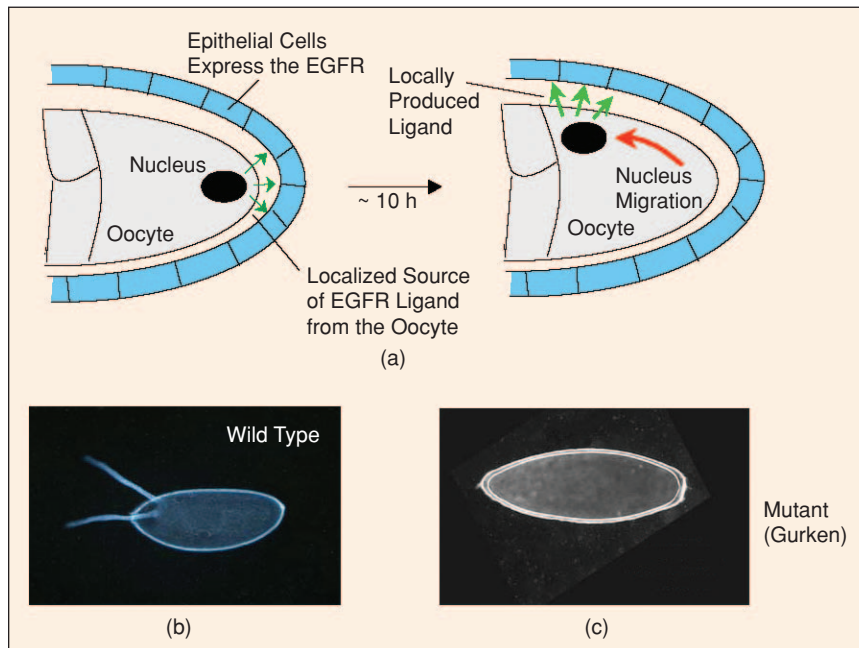
apical surface, while receptors are restricted to the basolateral part of the membrane [see Figure 2(a)]. In the intact epithelial layer, the ligands and receptors are kept apart, and, thus, the receptors remain inactive. When the epithelium is damaged, however, the apically secreted ligand induces cell division by activating receptors on the basolateral surface. The tight junctions between the newly formed cells re-establish the barrier between heregulin and its receptors [see Figure 2(b)]. In this way the wound is sealed and the receptor-mediated repair mechanism is reset to its initial off state.

Another well-studied example of spatiotemporally controlled receptor activation is found in fruit fly development [8], [9]. Both the anterior-posterior (AP) and the dorsoventral (DV) axes of the fruit fly (*Drosophila melanogaster*) embryo are specified during egg development. The mature eggshell is a three-dimensional (3-D) structure with a clear anterior-posterior and dorsoventral polarity [see Figure 3(b)]. The dorsal side of the eggshell is flatter than the ventral side; the anterior side is easily recognized by the presence of two dorsal appendages. The embryo develops inside the eggshell, and the appendages supply the developing embryo with oxygen. Importantly, the axes of the egg coincide with the axes of the embryo and the main body axes of the adult fly.

The axes of the egg and, hence, the axes of the future embryo are specified during egg development as a result of the interaction between the oocyte and the overlying layer of epithelial follicle cells. This interaction is mediated by the localized release from the oocyte of the EGFR ligand Gurken.

Locally produced ligand activates EGF receptors that are uniformly expressed in the follicular epithelium. The AP (front-back) axis is established when Gurken is secreted from the posterior cortex of the oocyte. Later in egg development, the same signal released from the dorsal-anterior cortex of the oocyte initiates DV (top-bottom) patterning of the eggshell and the embryo. In this system, the release of EGFR ligand is dynamically redirected from the posterior to the dorsal-anterior part of the cell as a result of regulated nuclear migration and localized mRNA processing. Genetic disruption of the oocyte-derived Gurken signal destroys both the AP and the dorsal-ventral asymmetries of the eggshell [see Figure 3(a)]. Thus, localized ligand secretion from the oocyte dictates different cell fates in the follicular epithelium.

These two examples from EGFR signaling in tissue homeostasis and development illustrate the sophistication of the extracellular level of control of receptor activation. Currently, this extracellular level is less understood than the intracellular signal transduction cascades induced by the activated receptor [10]. One reason



**Figure 3.** Autocrine signaling in morphogenesis. (a) Eggshell patterning and embryonic axes specification in the fruit fly *Drosophila melanogaster* is controlled by the EGFR-mediated interaction between the oocyte and the overlying follicular epithelium. The localized production of the EGFR ligand from the oocyte induces multiple cell fates across the follicular epithelium in *Drosophila* egg development. EGFR is uniformly expressed on the surface of the follicle cells. (b) Mature egg of *Drosophila melanogaster* is a three-dimensional structure with clear dorsoventral (top/bottom) and anteroposterior (front/back) polarities. (c) Genetic disruption of the EGFR-mediated interaction between the oocyte and the follicular epithelium can erase the axes of the egg.

for this disparity in understanding is that some of the molecules involved in ligand release and processing have been identified only recently. The other reason is that even the basic control variables of receptor-mediated mechanisms, such as ligand concentrations and receptor levels, cannot be reliably measured in tissues. To overcome this problem, we have developed models of autocrine EGFR signaling systems and used them to estimate the spatial operation of autocrine signals and the effects of autocrine signaling on the dynamics of intracellular signal transduction. Two of these models are described in the following sections.

### Random Walks of Autocrine Ligands and Spatial Localization of Autocrine Loops

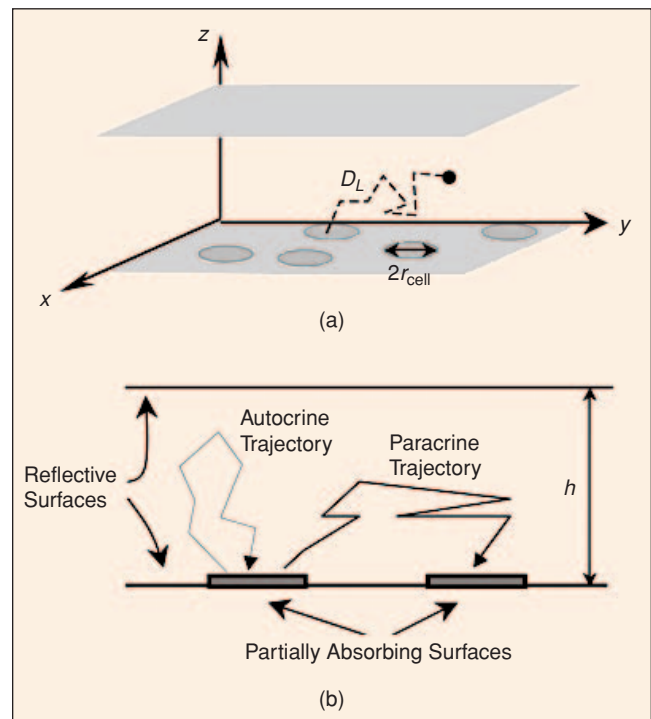
Autocrine signaling accompanies all stages of embryonic development and is important for tissue homeostasis. Amplified autocrine signaling has been associated with cancer. Mechanistic understanding of autocrine systems is important for manipulating them in tissue engineering or medicine. In tissues, autocrine loops are under the control of tissue architecture, cell density, and the developmental state of the cell. While it is impossible to control all of these variables in vitro, experiments with cultured cells can be used to pose fundamental questions about the operation of autocrine systems. For instance, cell culture experiments were used to analyze the spatial operation of autocrine loops [11]–[15]. Depending on the application, it can be useful to estimate the fraction of the ligands recaptured by the cell or the spatial distribution of the points at which escaping ligands are captured.

By studying the migration of epithelial cells plated at low cell density and displaying an active autocrine EGFR loop, we found that autocrine loops can operate at the level of a single cell [16]–[18]. At the same time, the escaping fraction of autocrine ligands can mediate cell-cell interactions. In particular, a growing number of experiments suggest that secreted growth factors contribute to the radiation-induced bystander effect, a phenomenon whereby radiation affects cells not directly hit by the radiation itself [19]. These studies naturally lead to questions about the spatial range of autocrine signals in cell culture assays.

In [20], we develop a simple stochastic model of autocrine loops in cell culture assays [see Figure 4(a) and (b)]. We model a two-dimensional (2-D) dispersion of cultured cells by the 2-D distribution of nonoverlapping disks of equal radius. A finite layer of medium for liquid diffusion covers the cells. Within the framework of this model, we analyze the probability of ligand recapture by the initial cell and the spatial distribution of the trapping points for the trajectories of the escaping ligands [see Figure 5(b)]. Our results for the distribution of the ligand capture points are derived as a function of measurable parameters of the cell, such as the cell surface density of receptors, and the

rate constants of binding and internalization and parameters of the assay, such as cell density and the height of the medium. For example, over a wide range of parameters, the fraction of trajectories recaptured by the cell can be found as a simple function of the dimensionless Damköhler number, which combines the cell surface density of receptors, the diffusivity of a ligand, and the linear dimension of the cell, given by  $Da \equiv k_{\text{on}} R_0 r_{\text{cell}} / D_L$ , where  $R_0$  is the cell surface density of receptors,  $r_{\text{cell}}$  is the radius of a disk approximating the cell,  $k_{\text{on}}$  is the forward binding rate constant, and  $D_L$  is the ligand diffusivity in the extracellular medium [see Figure 5(a)].

For the EGFR system, the forward binding rate constant is approximately  $10^8 M^{-1} \text{min}^{-1}$ . The Damköhler numbers corresponding to these values of  $\kappa$  lie between  $Da \approx 0.01$  for  $10^4$  receptors/cell and  $Da \approx 1$  for  $10^6$  receptors/cell. Using these values to calculate the probability of autocrine capture, we obtain  $P_{\text{au}} \approx 0.01$  for  $10^4$  receptors and  $P_{\text{au}} \approx 0.5$  for  $10^6$  receptors. Thus, 1% and 50% of ligand trajectories are recaptured by the cell in these two cases.



**Figure 4.** Models of ligand diffusion and binding in autocrine systems. (a) In our model of a cell culture assay, a random dispersion of cells is covered by a liquid layer of thickness  $h$ . The secreted ligand can be captured by receptors on the ligand-producing cell or its neighbors, giving rise to autocrine and paracrine trajectories, respectively. (b) Cells are modeled as randomly distributed disklike traps of radius  $r_{\text{cell}}$ . A reflecting boundary condition is placed at  $z = h$ . The boundary condition at  $z = 0$  is partially absorbing on the trap surface and reflecting otherwise.

## Positive Feedback in Autocrine Signaling

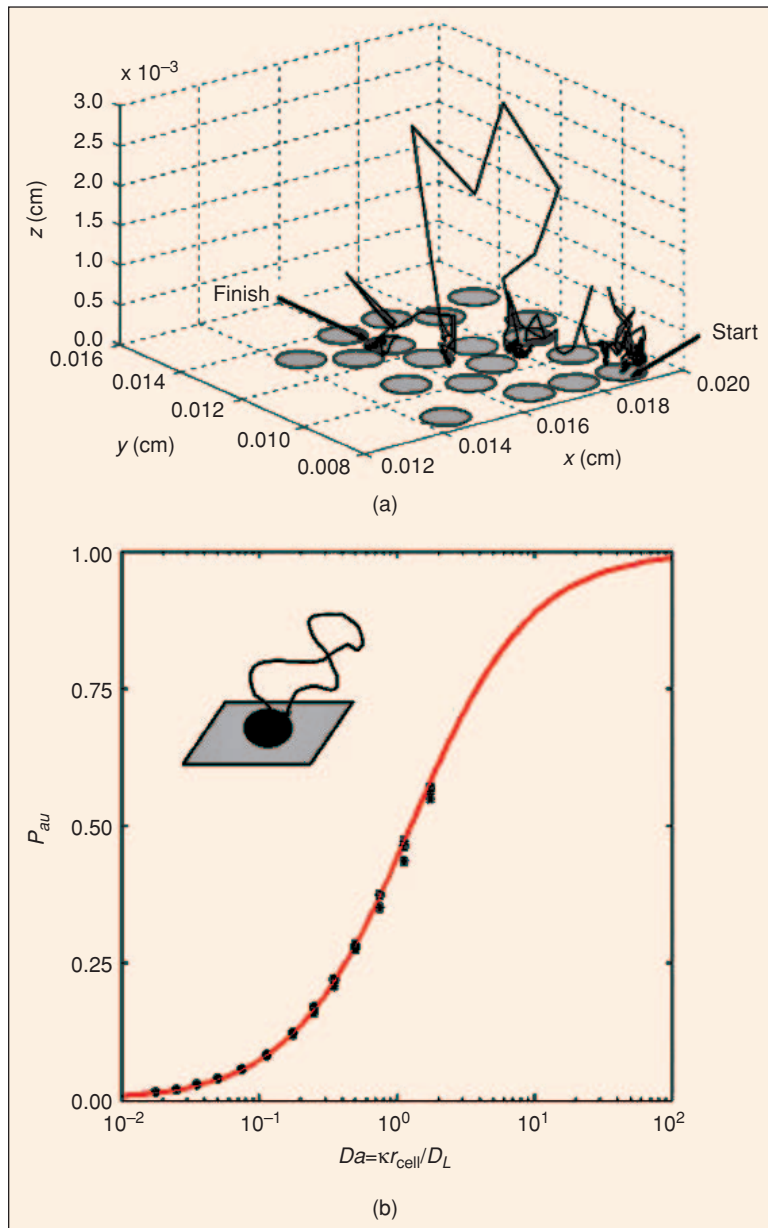
Recaptured autocrine ligands can activate signal transduction pathways in autocrine cells [see Figure 6(a)]. This autocrine signaling can stimulate further release of EGFR ligands, thus establishing positive feedback between ligand binding and ligand release. Stimulation of ligand release can depend on the EGFR-mediated transcription of EGFR lig-

ands or on the transcription of ligand-processing enzymes [21], [22]. Alternatively, processes that do not rely on gene expression can activate ligand release. For instance, signal transduction through the MAPK pathway can activate enzymes that process EGFR ligands into biologically active soluble forms. A control-oriented introduction to the MAPK pathway can be found in the recent review [23].

In embryogenesis, positive feedback in EGFR signaling is frequently involved in networks that pattern the developing tissues [24]. At the same time, positive feedback in the EGFR system has been identified in multiple cancers. In particular, it has been found that autocrine EGFR signaling protects cells from radiation-induced cell death. This effect is apparently mediated by a positive feedback loop between EGFR ligand binding and release. Because of these processes, the dynamics of the EGFR system must be considered during the design of cancer radiotherapies [25].

EGFR interferes with radiation-induced cell death, in part, by modulating the dynamics of radiation-induced MAPK signaling, which is a critical regulator of cell cycle progression. In several autocrine carcinomas, it was found that a 1–2 min pulse of ionizing radiation induces a MAPK response lasting for several hours, mediated through the EGFR [see Figure 6(b)]. Experiments with receptor blockade and extracellular medium transfer reveal that complex MAPK transients result from autocrine EGFR ligand secretion and capture. Significantly, it has been discovered that the inhibition of the long-lasting MAPK response enhances radiation-induced cell death in several carcinoma cell lines [25]–[27].

Quantitative analysis and modeling of EGFR involvement in radiation-induced cell death can potentially guide the design of effective cancer radiotherapies. Our initial model of EGFR-mediated radiation responses [28] accounts for the dynamic interaction between ligand release, extracellular transport, binding to cell surface receptors, endocytosis, and intracellular signaling through the MAPK cascade [see Figure 7(a)]. In this model, the variables correspond to free and occupied surface receptors, the activity of ligand-releasing proteases, extracellular endogenous ligand, and a three-stage enzymatic cascade, mimicking the MAPK module. Mechanistic blocks in the model are formulated on the basis of the extensive literature on EGFR binding, trafficking, and signaling. The modeling of ligand release is based on kinetic measurements of activation-induced degradation of ligand-releasing enzymes. In the absence of more detailed infor-



**Figure 5.** Brownian dynamics simulation of ligand diffusion and binding. (a) A representative trajectory generated by the Brownian dynamics algorithm [20]. (b) Analysis of autocrine trajectories. The autocrine fraction depends on the dimensionless group  $Da \equiv K_{on}R_0r_{cell}/D_L$ , which combines the size of the trap, ligand diffusivity, and the rate constant on the trap surface. The results of simulations are shown by symbols; the solid curve is given by  $P_{au} = Da/(Da + 4/\pi)$ .

mation, the interaction between mechanistic blocks is modeled by linear gains. For example, it is assumed that the rate of activation of the ligand-releasing enzyme is linearly proportional to the activation of the MAPK pathway.

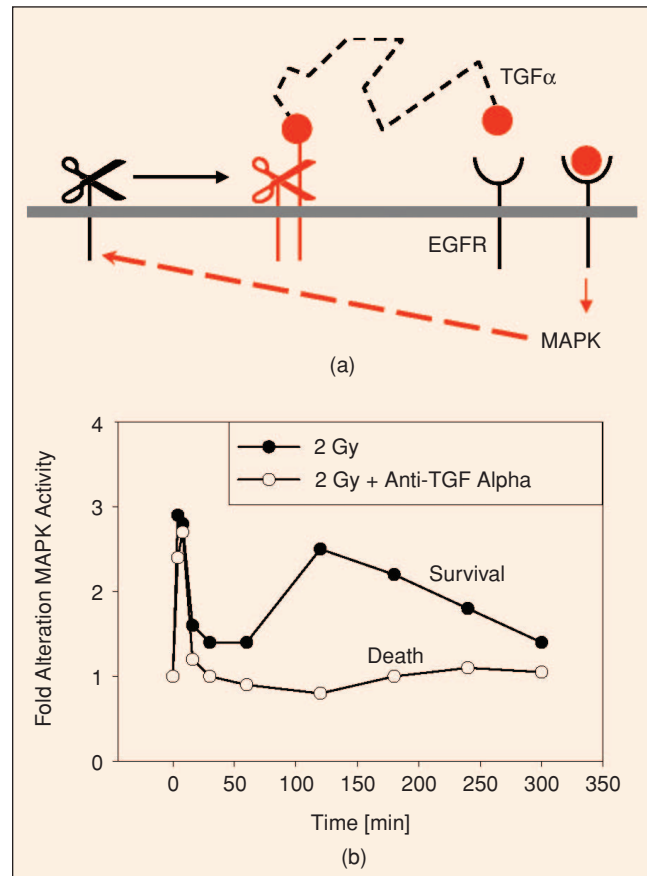
According to our model, secondary excitations in the MAPK radiation responses arise from strongly nonlinear interactions between ligand release, transport, binding, and signaling. While the time scale of each of the modules is less than one hour, their interaction can produce transients lasting several hours [see Figure 7(b)]. Our model successfully describes the qualitative changes in the signaling patterns due to quantitative variations in parameters characterizing the individual blocks. In particular, the model in [28] captures the qualitative differences in MAPK responses observed in cell lines expressing different EGFR receptors numbers [27] [see Figure 7(b) and (d)]. These results are directly correlated with the results of pharmacological inhibition of molecular components in the EGFR circuit. The model also predicts that the composition of the extracellular medium critically affects the duration and amplitude of intracellular response to extracellular stimuli. This prediction was confirmed experimentally by qualitatively changing the response upon addition of antiligand antibodies to the cell culture medium [see Figure 7(c) and (d)].

Almost every step of the radiation-induced EGFR activation is being intensively studied through a combination of biochemical and cell-based approaches. The existence of inhibitors for many steps in this network naturally leads to the question of how to choose the optimal combination of radiation dose and type as well as quantities of inhibitors. This selection can be greatly assisted by the further development of mechanistic models of radiation-induced cell signaling responses.

## Conclusions and Outlook

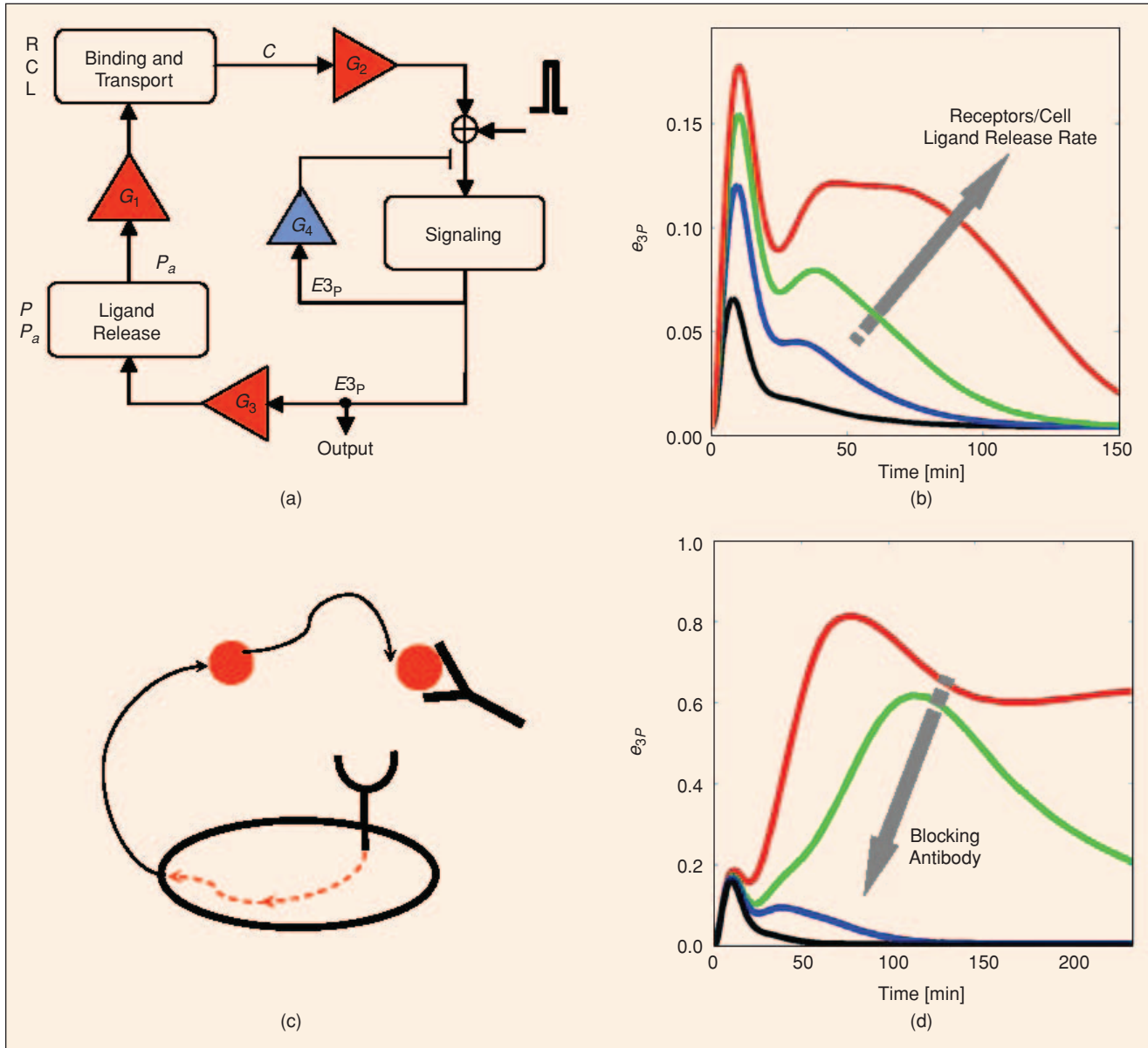
At this time, only a few dozen out of approximately 30,000 EGFR-related PubMed entries are dedicated to the modeling and computational analysis of EGFR signaling. Most models are based on molecular and cell-level processes. We need modeling at the tissue level, however, to understand how this system operates in vivo. Even the simplest models of EGFR signaling in tissues must simultaneously account for ligand release, transport, binding, intracellular signaling, and gene expression. Given this complexity, integrated models are nontrivial to test experimentally. Appropriate experimental paradigms that can be used to directly test predictions of complex models are critically needed. Cultured epithelial layers and model organisms used in developmental genetics, such as the fruit flies and nematodes, have shown great promise for achieving this goal.

Ideally, models developed for an experimental system should also predict the behavior of the same system under a different set of parameters as well as the behavior of related systems. For instance, models of EGFR signaling in *Drosophi-*



**Figure 6.** Complex dynamics of intracellular signaling induced by autocrine loops. (a) Positive feedback in the EGFR system relies on MAPK-mediated activation or expression of ligand-releasing proteases. (b) Secondary excitations in the radiation-induced MAPK dynamics depend on autocrine EGFR signaling in autocrine carcinoma cells. Secondary excitations in radiation-induced MAPK dynamics disappear when the autocrine loop is interrupted by the antibodies for the EGFR ligand (TGF $\alpha$ ).

la egg development should be of use in predicting EGFR signaling in patterning of the *Drosophila* wing. To make a single model useful across different cell lines, tissues, or even species, we must develop quantitative approaches to understand how EGFR signaling depends on the cellular and tissue context. To achieve this objective, it is necessary to develop quantitative assay for the interaction of EGFR with other signaling systems. Although much of the quantitative studies of the EGFR network have assumed that EGFR acts as an independent signaling module, it is highly unlikely that EGFR acts alone in mediating a specific cell or tissue response. Fortunately, an increasing amount of information on how the EGFR system interacts with other signaling pathways promises to help us understand how cells integrate signals from multiple pathways to produce a final response. Such understanding will bring us closer to being able to predict—and ultimately engineer—cell and tissue behavior.



**Figure 7.** Models of positive feedback in autocrine signaling. (a) The block-diagram of the mathematical model used to account for the secondary excitations in radiation responses. (b) Computational analysis of the model reveals that secondary excitations on a time scale of hours can arise as a result of autocrine signaling. (c) The model predicts that an intracellular signaling response to an exogenous stimuli can depend on the composition of the extracellular medium. (d) This prediction is verified in experiment: secondary excitation in radiation-induced MAPK dynamics disappears when the autocrine loop is interrupted by the antibodies that capture the EGFR ligand.

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