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Normal morphogenesis of epithelial tissues and progression of epithelial tumors

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Abstract

Epithelial cells organize into various tissue architectures that largely maintain their structure throughout the life of an organism. For decades, the morphogenesis of epithelial tissues has fascinated scientists at the interface of cell, developmental, and molecular biology. Systems biology offers ways to combine knowledge from these disciplines by building integrative models that are quantitative and predictive. Can such models be useful for gaining a deeper understanding of epithelial morphogenesis? Here, we take inventory of some recurring themes in epithelial morphogenesis that systems approaches could strive to capture. Predictive understanding of morphogenesis at the systems level would prove especially valuable for diseases such as cancer, where epithelial tissue architecture is profoundly disrupted.

Keywords

Morphogenesis; cancer biology; epithelial cells

Epithelial tissues define the boundary that separates our bodies from our world. They protect us from microbial pathogens and secrete proteins that help to digest our food and feed our young. Epithelia absorb nutrients from what we consume and excrete waste to detoxify our bloodstream. Together, epithelial cells bear the brunt of physical-chemical insults imposed by our environment, and consequently epithelial tumors (carcinomas) comprise roughly 90% of all human cancers.

To achieve their breadth of function, epithelial tissues have evolved elaborate and diverse cellular architectures. The regulated interplay of epithelial form and function is part of a more-overarching question of tissue morphogenesis. Namely, how do precise multicellular structures arise during development or remodeling, and how do these structures become disrupted during disease? Answering this question is complicated, because many factors are involved that span molecular, cellular, and tissue scales.

Due to its multifaceted regulation and hierarchical organization, epithelial morphogenesis should be fertile ground for contributions from systems biologists. However, in the decade since “systems biology” was coined,¹ morphogenesis has received little attention from a systems perspective. Perhaps this is because it is more difficult to break down the process into its fundamental units, as is commonly done with signaling pathways or gene-expression circuits.²

In this Advanced Review, we seek to define some of the core processes and modules that are evoked repeatedly during the morphogenesis of different epithelial tissues. Our focus is on mature epithelia because of their link to cancer. Thus, we do not discuss early

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morphogenetic programs in development, such as formation of the neural tube and ventral furrow, where transient epithelial cell states are involved. Another caveat is that the mechanisms drawn from the literature are not guaranteed to generalize across all epithelial tissues. We will therefore be careful to note the specific cell type used in each study. Nevertheless, the hope is that by organizing the biology of epithelial morphogenesis in this way, we can begin to define themes that apply to multiple epithelial-cell types.

We start with the basic morphologies that epithelial tissues can adopt, and then follow with the major inputs and outputs that drive their organization. Next, we discuss how epithelial morphologies and patterns are disrupted at the single-cell and tissue levels during tumorigenesis. We conclude with sections dedicated to experimental and computational models that have helped guide our understanding of epithelial morphogenesis.

Epithelial morphologies

Epithelial cells show characteristic organization at multiple length scales. We introduce two-dimensional epithelial cell sheets by using apico-basal and planar-cell polarity proteins as intracellular landmarks. We then discuss cellular and molecular pathways implicated in higher-order organization of epithelia into three-dimensional tubes, branches, and acini.

Sheets

Most normal epithelia exist as a thin sheet that displays asymmetry along the apical-basal axis (Figure 1(a)). The localization of ion channels and pumps to either the apical or basolateral membrane of epithelial cells generates a cellular gradient to transport solutes across the tissue (reviewed by Ref 3).³ The asymmetric distribution of membrane proteins is maintained by apical junctional complexes, which prevent the free diffusion of proteins between apical and basolateral domains.⁴ Junctional components include Par3, the adherens junction complex (consisting of E-cadherins, α -catenin and β -catenin), the Scribble group proteins (Scribble, Dlg, and Lgl) and ZO-1.⁵⁻⁸ These core interacting proteins influence assembly of the apical junctional complex and participate in apical-basal cell polarity, which is regulated during the morphogenesis of epithelial sheets.⁹⁻¹¹

Epithelial cells also can show another type of polarity, called planar cell polarity (PCP; see Table 1 for a list of abbreviations used in this Review), which is orthogonal to their apical-basal axis and in the orientation of cell-cell and cell-extracellular matrix (ECM) contact (Figure 1(b)). Asymmetric localization of mammalian PCP genes, such as Vangl2, Celsr1 and Fz6, is required for proper anterior-posterior compartmentalization of follicles in hair cells.¹² The mechanisms of PCP regulation are being unraveled genetically through studies of bristle orientation in *Drosophila* epithelia. The signaling proteins of the core PCP group include frizzled (fz), disheveled (dsh), Van Gogh (Vang), prickly (pk), diego (dgo) and flamingo (fmi). During the development of the fly wing, the core PCP molecules are first evenly distributed near the adherens junction. Later, fz-dsh-dgo concentrate as a complex at the distal edge, whereas Vang-pk accumulate at the proximal sides. fmi co-localizes and interacts with both fz and Vang to form stable complexes.¹³

Amonlirdviman and coworkers built a local-feedback model encoding behaviors of PCP proteins that was sufficient to describe the local alignment of PCP complexes.¹⁴ However, this model has difficulty reconciling PCP mutant phenotypes in other contexts.¹⁵ A more in-depth biochemical understanding of the relationships between PCP proteins may be required to build computational models that are broadly predictive.

Another important morphology of epithelial sheets is the columnar shape of individual cells (Figure 1(c)). The regulation of cell shape in epithelial sheets is crucial for many facets of

morphogenesis. For example, the change in cell shape driven by apical constrictions is required for epithelial remodeling during tube formation of ventral furrow cells during *Drosophila* gastrulation.¹⁶ Columnar epithelial cell shape is controlled by two major mechanical forces: cortical tension and cell-cell adhesion.¹⁷ Cortical tension arises from the force generated within the cytoskeleton by actin-myosin-II interactions. Tension is exerted on the cortical F-actin network by myosin-II, which has been implicated in cell elongation during anaphase¹⁸ and in establishing the characteristic hexagonal shape of epithelial cells in proliferating intestinal epithelial cysts.¹⁹ Cortical tension is counter-balanced by adhesive forces from neighboring cells. When taken together, cells in an epithelium tend to minimize contact surface energy, resulting in a polygonal, cobblestone shape when viewed from above.²⁰ The principle of energy minimization also applies to the shapes that epithelial sheets will adopt.²¹⁻²³ Therefore, despite its simple architecture, the epithelial sheet is rich in cell and molecular organization that is important for proper function.

Tubes

Absorptive and secretory epithelia must arrange themselves macroscopically into tubes to provide conduits for directed transport within the organism. Tubes are comprised of polarized epithelial cell monolayers that surround a central lumen (Figure 2(a)). The apical epithelial surface lines the lumen, the basal surface faces the surrounding tissue and the lateral surface connects adjacent cells through intercellular junctions. Despite the often-specialized function of epithelial tubes in different tissues, tubulogenesis itself occurs in only a few ways.

Many epithelial tubes are formed by remodeling pre-existing polarized epithelial sheets.^{24, 25} Two related mechanisms of formation of epithelial tubes from polarized cells are wrapping and budding.²⁶ With wrapping, an epithelial sheet invaginates and curls, forming a final tube that is parallel to and separated from the surrounding epithelia, as during tube formation in the *C. elegans* digestive tract.²⁷ With budding, cells migrate out and form a new tube. The new tube extends orthogonally from the original epithelial sheet. Budding is the principal means for tubulogenesis in the *Drosophila* trachea.²⁸ Elsewhere, tubes form by polarization of epithelial precursors and exocytosis of vesicles or by removal of inner cells through apoptosis.^{24, 25} Unlike wrapping and budding, this mechanism requires initiation of a polarized epithelium. Polarization can be regulated both by ECM signals²⁹ and by cell division.^{30, 31} Recent *in vitro* studies have shown that Rab-mediated membrane traffic and polarity complexes can cooperate to generate the apical surface and lumen *de novo*.³² Directional trafficking works both upstream and downstream of polarity proteins, suggesting a positive-feedback mechanism for generating polarity.

After cells have assembled into cylindrical cords, lumens can be created by eliminating cells in the center (cavitation) or by forming lumens *de novo* at apical membrane sites between cells (cord hollowing).²⁶ Cavitation occurs, for example, in mice during the formation of submandibular and mammary glands^{33, 34} and will be further discussed below. Cord hollowing occurs in rapidly polarizing Madin-Darby canine kidney (MDCK) cysts,³⁵ during formation of the zebrafish gut,³⁶ and during pancreatic tubulogenesis.³⁷ In the zebrafish gut, small lumens then combine into a single lumen by a process that involves paracellular ion transport.³⁶ Single lumen specification is genetically regulated by the expression of ion transporters, which control luminal fluid accumulation and promote the coalescence of multiple small lumens.

Branches

Tubular networks are joined at bifurcations that initiate as branch points during organ development (Figure 2(b)). Branching morphogenesis is a process whereby the epithelium

elongates against the stroma or mesenchyme. Epithelial branches can be achieved by very different strategies depending on the tissue and the organism. Most commonly, branching involves cell proliferation together with cell migration, and both processes will be discussed below. However, tracheal branching in *Drosophila*³⁸ and submandibular gland branching in mice³⁹ do not require proliferation. Conversely, the early stages of lung branching in mammals occur normally even when migration is severely impaired.⁴⁰ Thus, branched tissues may exploit proliferation or migration depending on the context, but neither appears universally required.

Ewald and coworkers used long-term confocal imaging and organotypic breast-epithelial culture techniques to visualize branching morphogenesis *in vitro*.⁴¹ In this culture model, branch morphogenesis initiates from the sprouting of highly proliferative terminal end buds (TEBs) of the mammary gland. TEBs extend from a duct and are partially depolarized. During branch elongation, junction-connected luminal epithelial cells advance collectively, whereas loosely connected myoepithelial cells appear to restrain elongating ducts. Thus, elaboration of the mammary ductal tree may result from antagonism between advancing TEBs and a belt of myoepithelia that locally constricts elongation.

Mechanical stress transmitted by the ECM has also been implicated in branch formation. For example, ECM degradation in embryonic mouse lung enables the adjacent epithelium to invade as a bud.⁴² Migratory epithelial cells and invasive cancer cells have been reported to generate surface-localized protease membrane type 1 matrix metalloproteinase (MT1-MMP), which causes degradation of local pre-existing ECM and reduces mechanical resistance.^{43, 44} Tumor-associated myoepithelial cells, which contact the basement membrane directly, deposit additional basement membrane components around the duct to help maintain the polarity of luminal epithelial cells after branch formation.⁴⁵ ECM regulation thus plays an important role in epithelial branching.

Cell-cell and cell-ECM interactions are complemented by diffusible growth factors that act as instructive chemotactic cues for branch formation. Glial cell line-derived neurotrophic factor (GDNF), epidermal growth factor (EGF), and fibroblast growth factor (FGF) drive kidney branching,⁴⁶ whereas EGF and hepatocyte growth factor (HGF) are critical branching factors in the mammary gland and lung.^{47, 48} By contrast, transforming growth factor β (TGF β) is a potent inhibitor of branching in luminal epithelia.⁴⁹ Nelson and colleagues used micropatterning together with computational modeling to reveal that branches form at sites of low local concentration of inhibitory autocrine morphogens, such as TGF β .⁵⁰ These results illustrate how tissue geometry controls the local microenvironment to regulate branching morphogenesis.

Acini

Branched epithelial trees terminate in secretory or absorptive epithelial acini that increase surface-to-volume ratios of the organ (Figure 2(b)). Their formation typically requires an explicit cavitation process to convert proliferating buds into mature acini (Figure 2(c)). Cavitation starts with the polarization of cells on the periphery, followed by lumen clearance of cells in the center. Cavitation is observed in 3D models of mammary acini (MCF10A cells),^{51, 52} in kidney cell-culture models (MDCK cells),³⁵ and also in mouse TEBs *in vivo*.³⁴ Interestingly, both cavitation and cord hollowing occur in MDCK cells, depending on cell polarization efficiency,³⁵ suggesting that cavitation may act as a backup hollowing mechanism for epithelia that polarize slowly during morphogenesis.

Clearance of the lumen during cavitation is strongly influenced by the pro- and anti-apoptotic proteins of the Bcl-2 family. For example, overexpression of Bcl-2 or Bcl-X_L inhibits caspase-3 activation and delays lumen formation in 3D breast epithelial cultures.⁵¹

Bim, a key Bcl-2 sensor of integrins and EGF receptor deprivation, is induced after cell detachment and contributes to programmed cell death in MCF10A 3D culture.⁵³ Inhibition of Bim expression significantly delays apoptotic cell death of the central cells,⁵⁴ and *in vivo* disruption prevents apoptosis and delays lumen clearing in mouse TEBs.³⁴ Functional loss of another BH3-only protein, Bmf, provides protection from cell death during MCF10A anoikis and 3D morphogenesis.⁵⁵ Thus much of Bcl-2-family regulation associated with apoptotic clearance is linked to stress caused by ECM detachment. However, programmed cell death is not the only mechanism for lumen clearance because cavitation eventually takes place even when apoptosis is blocked. Upregulated prodeath ligands such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) have been shown to cause autophagy in ECM-deprived cells.⁵⁶ Therefore, clearance may involve a mixed cell-death program that ensures complete cavitation.

Epithelial cell outputs

Epithelial tissue morphology arises from the phenotypic responses of individual cells. These cellular “outputs” organize into major categories that control cell orientation, its microenvironment, and overall population size.

Proliferation and apoptosis

As with nearly all tissues, total cell numbers are tightly regulated during the development and maintenance of epithelia. The expansion, contraction, and homeostasis of epithelial cell populations are determined by the rates of cell proliferation and cell death. Recent work suggests that the timing and location of these events is perhaps more critical than the quantitative extent of proliferation or death during epithelial morphogenesis. For example, during *in vitro* mammary branching morphogenesis, epithelial proliferation is transiently increased in the vicinity where new ducts have initiated.⁵⁷ If proliferation is constitutively high, then branching morphogenesis is suppressed and epithelia grow as spheroids.⁵⁸

Similar results have been reported for apoptotic cell death during breast-epithelial acinar morphogenesis.⁵² Apoptosis is a critical event during luminal clearance of epithelial spheroids, and cell-death inhibition synergizes with hyperproliferation-inducing oncogenes to cause luminal filling.⁵¹ However, apoptosis must also be suppressed during the preceding phase of proliferation and among matrix-attached cells that eventually form the final acinar structure. Yerbes *et al.* recently demonstrated the tight requirement for apoptosis control by perturbing the levels of c-FLIP, an antiapoptotic protein that is transiently induced during morphogenesis.^{55, 59} As expected, c-FLIP overexpression caused delayed luminal clearance in a manner similar to that reported for Bcl-2 overexpression.⁵¹ But surprisingly, the authors found that stable knockdown of c-FLIP prevented acinar formation altogether,⁵⁹ suggesting that apoptosis must first be suppressed and then selectively induced to give rise to normal acinar morphology. These findings together suggest that a systems-level understanding of morphogenesis will be aided by computational models that explicitly consider the timing of cell outputs.^{60, 61}

Extracellular matrix remodeling

Epithelia are kept separated from the adjacent tissue stroma by a special form of ECM called basement membrane. The removal and deposition of basement membrane is critical for epithelial branching morphogenesis.⁶² Basement membranes are highly self-assembled sheets in which glycoproteins and proteoglycans are cross-linked to laminins and collagen IV fibrils along the basal side of epithelial cells. Basement membrane degradation is catalyzed by metalloproteinases, including the matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin repeats (ADAMTS) families. The

local composition, turnover, and mechanics of basement membrane together govern tissue organization.

Membrane-tethered MMPs (MT-MMPs) play an especially important role in branching morphogenesis.⁶³ MT1-MMP degrades fibrillar collagen as an initial cue for cancer cell migration.⁴⁴ MT2-MMP-dependent proteolysis of collagen IV is linked with epithelial proliferation to coordinate the morphogenetic program of the submandibular gland.⁶⁴ ADAMTSs act as the dominant secreted metalloproteinases cleaving large chondroitin-sulfate proteoglycans during morphogenesis.⁶⁵ For example, three ADAMTS family members, Adamts5, Adamts9, and Adamts20 cooperate in limb morphogenesis to influence cell apoptosis and the regression of interdigital tissues.⁶⁶ The tethering of MT-MMPs allows basement membrane degradation to be localized to individual cells, whereas ADAMTSs may require diffusion from the cell surface to gain access to their bulky substrates.

In addition to ECM degradation, myoepithelial cells in breast tissue secrete basement membrane components (including laminins, nidogen 1, perlecan and type IV collagen) between the cells and the ECM to form the secondary ECM around the new branch.⁶⁷ Myoepithelial cells also produce maspin, an inhibitor of ECM-degrading proteases.⁶⁸ Thus, epithelial tissues can upregulate basement membrane constituents by active secretion or inhibition of turnover.

Single-cell migration

Single-cell migration is complex and heterogeneous during morphogenesis. Epithelial cells migrate along a basement membrane, not through interstitial tissues, and often stop upon terminal differentiation. Yet, most cell migration involves the cyclic repetition of the same steps: edge protrusion, interaction with the ECM, translocation of the cell body, and retraction of the cell rear in response to ECM ligands.⁶⁹ Individual cells transiently form and resolve cell-cell contacts during single-cell migration, whereas collective migration occurs with stringent cell-cell adhesions that silence migration activity of cells within the group. Cell-cell junctions are tightly controlled during migration and this control can be regulated by other factors. For example, TGF β activation is associated with the loss of cell-cell adhesions and single-cell movement in breast cancer, while the inhibition of TGF β signaling reverts these cells to collective migration.⁷⁰

Single-cell migration in embryonic development usually involves an epithelial-to-mesenchymal transition (EMT), but dedifferentiation is rare in maturing tissue. Nevertheless, singly motile cells are often observed within epithelial tissues, such as during branching morphogenesis of ureteric buds and salivary glands,^{71, 72} tissue remodeling of the small intestine, and polarization of breast epithelial acini.^{57, 73, 74} The migration of single epithelial cells occurs within a larger group of nonmotile epithelia and is typically not coordinated with other cells that happen to be moving at the same time. However, “orchestrated” single-cell rearrangements have been documented in the Wolffian duct epithelia of the ureteric bud and in engineered mammary epithelial tubules.^{75, 76} Currently, there is a lack of molecular tools that can target single-cell migration without affecting collective migration or other processes involving the cytoskeleton. Thus, although single-cell migration is undoubtedly an important phenotype, its precise quantitative role during morphogenesis is difficult to define.

Collective cell migration

Collective cell migration is distinct from single-cell migration in that cells in the same group remain connected with cell-cell junctions and coordinately exhibit front-rear polarity during movement.⁷⁷ Among cells at the leading edge, force generation occurs by engagement of

integrins with ECM at the free edge and cadherin-mediated cell-cell junctions with neighboring cells. Cell-generated contractile forces are regulated by a “tug-of-war” between cell-cell and cell-matrix adhesions during sheet migration in 2D and branching in 3D, both of which involve collective mechanisms. For example, cadherin cell-cell contacts in MCF7 breast epithelial monolayers are modulated by translocation of the focal adhesion protein vinculin from cell-matrix adhesions to cell-cell junctions.⁷⁸ Conversely, increasing matrix stiffness elevates Rho kinase (ROCK)-generated contractility and focal adhesion formation in mammary epithelial cells cultured in 3D matrix, which in turn weakens adherens junctions and disrupts morphogenesis.⁷⁹

Various epithelial tissues restore their sheet-like organization by collective migration during wound healing.^{80, 81} Cell migration at the healing front is coupled with epithelial proliferation within the sheet during wound closure. Mechanical force is a prominent factor in collective 2D migration. Leading cells initiate lamellipodia into the wound area, which transmit force to follower cells by Rho-dependent mechanisms.^{82, 83} When the leading cells at the front edge extend protrusions, cells away from the wound edge form basal membrane protrusions, which together generate the main traction forces for motility.⁸⁴ Thus, collective 2D migration does not originate exclusively from the “pulling” of the leading cells at the wound edge or the “pushing” of proliferating cells away from the edge, but by the tensile stresses that accumulate globally in the tissue.⁸⁵

In 3D, collective migration is a dominant mechanism of cellular reorganization during tube elongation and branching morphogenesis. In the zebrafish lateral line primordium and *Drosophila* trachea, a group of leading cells guides a strand-like collective stalk during branching.^{86, 87} Collective migration occurs without leading cell protrusions, such as filopodia and lamellipodia.⁵⁷ Developing tissues often further require proteolytic mechanism to remove ECM barriers.⁸⁸ To date, much of the work in 3D migration has focused on the importance of diffusible factors. In the trachea, branching by collective migration is initiated in cells at the tip of each branch upon stimulation with FGF.⁸⁹ FGF-induced collective migration has also been implicated in branching of primary organotypic 3D cultures of mouse mammary glands. Notably FGF also promotes canonical 2D wound healing in epithelial sheets,⁹⁰ suggesting that the same factor may promote collective migration differently in 2D-vs-3D contexts.

Cell polarization

In 3D *in vitro* cultures of MDCK epithelial cysts, apicobasal polarity of mature epithelia is established before cell division completes. A discrete apical surface develops between dividing daughter cells after spindle orientation has been established by the Rho-family GTPase, Cdc42.⁹¹ Cdc42 together with Rac1 (another small G protein) are thought to determine the early steps in epithelial polarization. Rac1 and Cdc42 are essential during the establishment of apicobasal polarity in MDCK cysts.^{92, 93} In response to cell-matrix interactions, β 1-integrins orient the apical pole of polarized cysts via Rac1 activation.²⁹ In 3D cultures of an intestinal epithelial cell line (Caco-2), Cdc42 regulates spindle orientation and apical surface positioning during cell division.⁹¹ Rich1, a Cdc42-specific GTPase-activating protein (GAP), associates with components of the apical-polarity network and maintains tight-junction assembly between MDCK epithelial cells through its regulation of Cdc42.⁹⁴ Conversely, the Cdc42-specific guanine nucleotide exchange factors (GEFs), tuba and intersectin2, serve as key activators of Cdc42 to control spindle pole orientation during cyst formation.^{95, 96}

The initial cell-cell adhesion and cell-ECM contacts provide the first spatial cues for cells to distinguish apical from basolateral surfaces. For instance, in MDCK cells the post-Golgi vesicles containing basolateral membrane proteins are targeted directly to the site of E-

cadherin-mediated cell-cell contacts, while apical proteins are excluded.⁹⁷ In addition, cell-ECM contacts, such as those between collagen I and $\beta 1$ -integrins, can prime basement membrane deposition by Rac1-dependent mechanisms to reinforce cell polarity.²⁹ This cell-ECM priming is critical, because apicobasal polarity inverts when $\beta 1$ -integrin or Rac1 function is blocked.^{29, 92} Interestingly, polarity inversion in MDCK cells is not a default pathway but instead is regulated by another GTPase, RhoA, along with its effectors, ROCK1 and myosin II.⁹⁸ A morphologically similar inversion of glandular epithelial polarity has been detected during tumor progression,⁹⁹ suggesting cells may occasionally lack the appropriate priming ECM *in vivo*.

Although stable in mature tissues, apicobasal polarity is dynamic and heterogeneous during morphogenesis and tissue remodeling *in vitro*. For example, cells within the TEB-like region of cultured mammary glands partially lose apicobasal polarity during expansion and branching.⁵⁷ Apicobasal polarity is also transiently downregulated during HGF-induced tubulogenesis in MDCK cysts.¹⁰⁰ The dynamics of cell polarity link tightly to the proliferative and differentiation states of epithelial cells.¹⁰ Thus, cell polarity should be considered among the earliest epithelial outputs, setting the stage for many phenotypes critical for morphogenesis.

Epithelial cell inputs

The precise signature of epithelial cell outputs during development is determined by a host of extracellular factors that act as “inputs” to shape cell fate and tissue organization. Growth factors and cytokines diffuse through tissues to exert long-range control, whereas paracellular signals are sent by an epithelial cell’s immediate neighbors. In addition, mechanical inputs are now widely recognized to influence cell fate and tissue function.¹⁰¹ When possible, we focus on systems models and systems experiments in the literature that have focused on the factors known to be important for epithelial morphogenesis.

Diffusible factors

Hepatocyte growth factor—Hepatocyte growth factor (HGF) is a fibroblast-derived factor that promotes motility, proliferation, and tubule formation in epithelial cells.^{102, 103} HGF binds to the receptor tyrosine kinase (RTK) proto-oncogene c-Met, leading to receptor dimerization and autophosphorylation.^{104–106} c-Met tyrosine phosphorylation recruits multi-protein docking complexes containing growth factor receptor binding protein 2 (Grb2) and son-of-sevenless homolog protein (SOS) that promote Ras–MAPK activation (Figure 3(a)).^{107, 108} Additionally, activated c-Met dimers bind directly to Grb2-associated binding protein 1 (Gab1), which induces downstream epithelial cell motility through its interaction with the Crk-like adaptor protein.^{109, 110} Gab1 also serves to recruit phosphoinositide 3-kinase (PI3K), resulting in lipid signaling that activates the anti-apoptotic AKT pathway.^{111, 112}

The HGF-c-Met signaling pathway has been examined computationally with logic-based Boolean networks that reflect published protein-protein interaction data.¹¹³ By applying this model to microbial pathogenesis, HGF-induced pathways were fused with *H. pylori* signaling to examine c-Met activation during infection. Boolean approaches thus provide a convenient means for combining diverse signaling sub-networks, as has recently been shown in hepatocytes.¹¹⁴

Fibroblast growth factor—The fibroblast growth factor (FGF) signaling pathway contains 22 known ligands and four FGF receptors (FGFRs), leading to a large functional diversity for the signaling network.^{115, 116} FGF signaling plays a crucial role in epithelial morphogenesis as an inducer of branching and differentiation in lung epithelia.¹¹⁷ Under

normal conditions, FGF is sequestered within the ECM and requires the presence of heparin sulfate proteoglycans (HSPGs) to bind to the appropriate FGFR and initiate signaling.¹¹⁸ HSPGs act as reservoirs for FGF ligands promoting their dimerization to induce higher-affinity FGFR binding, receptor dimerization, and RTK activation.¹¹⁶ Dimerized FGFRs phosphorylate and bind fibroblast growth factor receptor substrate 2 (FRS2), which recruits the Grb2–SOS complex leading to Ras–MAPK pathway activation (Figure 3(a)).^{119, 120} FGF ligand binding to FGFR additionally causes FGFR ubiquitylation, Src-mediated endocytic trafficking, and receptor downregulation.^{121–123}

Like many RTKs, FGFR contains multiple tyrosine residues that become autophosphorylated during activation. The precise sequence of these autophosphorylation events is difficult to characterize but is important for quantitative modeling of the overall signaling network.¹²⁴ To address this, Furudi *et al.* developed an approach for FGFR autophosphorylation that combines native gel electrophoresis with mass spectrometry.¹²⁵ The authors used native gels to determine the stoichiometries and kinetics of tyrosine phosphorylation together with mass spectrometry to identify specific phosphotyrosine residues. By fitting their data to a kinetic model, Furudi *et al.* found that the first tyrosine is phosphorylated the fastest, with each subsequent phosphotyrosine accumulating at half the rate of the one before it. These empirical differences in autophosphorylation kinetics have not yet been implemented in quantitative models of FGFR signaling.¹²⁶

Epidermal growth factor—Epidermal growth factor (EGF) is the founding member of a family of ligands that promote epithelial proliferation.¹²⁷ EGF and EGF-like ligands directly or indirectly bind to the epidermal growth factor receptors, ErbB1–4. Canonical EGF signaling is very similar to the RTKs introduced above: ligand binding causes receptor autophosphorylation, which creates binding sites for the adaptors Shc and Grb2–SOS (activating Ras–MAPK), and the p85 subunit of PI3K (activating AKT signaling through the p110 catalytic subunit of PI3K) (Figure 3(a)). Added complexities arise from the properties of the individual receptors—ErbB2 does not bind ligands and ErbB3 lacks tyrosine-kinase activity—and from their differing abilities to homo- or heterodimerize and traffic intracellularly upon activation.¹²⁸

Signaling downstream of ErbB receptors is the best characterized of all RTKs, rendering it ideal for computational modeling.¹²⁹ Various models have been built to emphasize specific facets of the biology, such as MAPK signaling emanating from different ErbB adaptor proteins,¹³⁰ ligand-specific ErbB activation and signaling,¹³¹ and crosstalk among ErbB-induced immediate-early genes.¹³² EGF-family signaling is therefore a prime candidate for future multiscale models that link molecular networks to multicellular organization.

Transforming growth factor β —Transforming growth factor β (TGF β) signaling plays a critical role in inhibiting cell proliferation and exerts homeostatic control of cell numbers in many epithelial tissues.^{133–137} TGF β ligands bind to a heterodimer complex of two receptor serine-threonine kinases, TGF β RI and TGF β RII. Upon ligand binding, the TGF β RII serine phosphorylates TGF β RI to activate the kinase,¹³⁸ leading to the subsequent phosphorylation of the SMAD transcription factors, SMAD2 and SMAD3 (Figure 3(b)).¹³⁹ Before TGF β R stimulation, SMAD2/3 proteins are sequestered in the cytoplasm, and upon phosphorylation, SMAD2/3 translocates to the nucleus with the co-SMAD, SMAD4, to initiate downstream transcriptional activity.¹⁴⁰ As with the RTKs, signaling activity is terminated by the ubiquitylation and downregulation of TGF β Rs through endocytic trafficking and subsequent lysosomal degradation.¹⁴¹ The exact mechanism of surface receptor regulation remains unclear, but it is an important aspect of TGF β R signaling, as constitutive internalization can entirely sequester TGF β Rs in the cytoplasm.¹⁴²

To examine the signaling properties conferred by TGF β R trafficking, Vilar and coworkers built a simple two-compartment model of the network.¹⁴³ The authors predicted that regulation of constitutive-vs.-ligand-induced TGF β R degradation in endosomes could determine transient-vs.-sustained SMAD responses to TGF β ligands. Validation of this prediction awaits quantitative measurements of TGF β R trafficking, like those available for the ErbB receptors.¹⁴⁴

Wnt—Wnt comprises a family of lipid-modified ligands that promote proliferation and differentiation of epithelial cells during morphogenesis.¹⁴⁵⁻¹⁴⁹ There are 20 different Wnt ligands in humans that bind to Frizzled (Fz) receptors and the co-receptor, low-density lipoprotein receptor-related protein (LRP).¹⁵⁰⁻¹⁵² In the absence of Wnt, the key effector protein of the pathway, β -catenin, is subject to constitutive proteolysis by a destruction complex composed of two scaffolding proteins (Axin and APC) and two kinases (GSK3 β and CK1) (Figure 3(c)).¹⁵³⁻¹⁵⁷ Binding of Wnt to Fz-LRP translocates the destruction complex to the plasma membrane along with a third scaffolding protein, disheveled (Dsh), which leads to inhibition of the destruction complex and the consequent destabilization of β -catenin levels in the cytoplasm.¹⁵⁸⁻¹⁶¹ Stabilized β -catenin accumulates in the nucleus and acts as a coactivator for TCF/LEF family proteins to induce gene expression.¹⁶²⁻¹⁶⁵ Thus, among diffusible factors important for epithelial morphogenesis, the Wnt pathway is unique in that protein localization and stability act as the major signal transducers, with phosphorylation playing a supporting role.

A quantitative differential equations-based model of the canonical Wnt pathway has been defined by biochemical reconstitution of the core signaling elements.^{166, 167} Using frog egg extracts to study the Wnt pathway in a test tube, the authors were able to avoid complications associated with protein localization in cells and focus on the phosphorylation-degradation reactions. The stability of β -catenin is directly controlled by phosphorylation, but it can also be indirectly modulated by the scaffold Axin, which is degraded in response to Wnt.^{168, 169} The model provided explanations for why scaffold destruction would be beneficial for Wnt signal transmission. More generally, it highlights classical biochemical approaches as important-but-underutilized tools for systems-biology research.¹⁷⁰

Paracellular factors

E-cadherin—Information is directly transmitted between neighboring cells by cell-cell signaling receptors. The major homotypic receptor in epithelia cells is E-cadherin, a Ca²⁺-dependent receptor that is critical for cell-cell recognition and adhesion (Figure 4(a)).¹⁷¹ The cytoplasmic domain of E-cadherin connects to the actin cytoskeleton through α - and β -catenin to preserve cell shape and polarity. In MDCK cells, cadherin-catenin complexes influence signaling predominantly through small GTPases. Rac-induced lamellipodia activity is suppressed as E-cadherin accumulates, and Rho-dependent actomyosin contractility increases during *de novo* contact formation once cytoskeletal connections have formed.¹⁷²

Loss of E-cadherin is sufficient to promote an EMT in breast epithelial cells.¹⁷³ EMT may promote metastasis of solid tumors,¹⁷⁴ and many EMT inducers directly or indirectly suppress E-cadherin expression.¹⁷⁴⁻¹⁷⁷ The dynamics of E-cadherin- β -catenin interactions and its regulation of EMT were incorporated into a multi-scale model of cell-cell adhesion.¹⁷⁸ Within this model, regulation of cell-cell adhesion by E-cadherin was sufficient to describe single-cell detachment from neighboring cells. Conversely, restoration of E-cadherin in invasive carcinomas has been shown to reduce the metastatic potential of transformed MCDK cells and human breast carcinoma cells.¹⁷⁹ Therapies that can restore E-

cadherin protein levels may be effective in cancers where E-cadherin transcription is suppressed.

Delta-Notch—Epithelial cells also signal to adjacent cells through heterotypic interactions involving the Notch receptor (Figure 4(b)). Notch binds to one of its ligands (Delta or Jagged) on a neighboring cell, causing proteolytic cleavage of the receptor. This cleavage releases an intracellular domain of Notch1 that then translocates into the nucleus and induces gene expression. Among Notch-dependent genes is a group of cyclic transcriptional repressors, called the Hairy/enhancer of split (Hes) family, which oscillate in expression by repressing their own transcription.¹⁸⁰ An additional layer of negative feedback is provided by the Notch-modifying glycosyltransferase enzyme, Lunatic Fringe, which is an inhibitor of Notch signaling and also a Notch target gene.¹⁸¹ These negative feedback loops are thought to contribute to the different responses of cells to varying levels of Notch stimulation. For example, in breast epithelial cells, high Notch activity suppresses cell proliferation and cell-ECM adhesion, whereas low Notch activity causes hyperproliferative acini and maintains matrix adhesion.¹⁸² By virtue of these dose-dependent actions, Notch acts as a morphogen during development, whose function may be subverted during tumorigenesis.¹⁸³

Computational models are investigating whether Delta-Notch negative-feedback loops are sufficient to generate the long-range patterns in signaling observed during development. Webb and Owen have compared several of the prevailing models that incorporate different non-cell autonomous properties of Delta-Notch signaling.¹⁸⁴ In some contexts, high Delta expression in one cell suppresses Delta expression in its neighbors through Notch by a process called lateral inhibition.¹⁸⁵ In other contexts, the reverse happens through a process called lateral induction.¹⁸⁶ Surprisingly, the authors found that both mechanisms are capable of generating long-range patterns for certain feedback strengths. Further computational work on Delta-Notch signaling could be used to identify perturbations to signaling or feedback that would distinguish whether lateral inhibition or lateral induction is the dominant non-cell autonomous mechanism.

Eph-Ephrin—Adjacent epithelial cells communicate bidirectionally through interactions between Eph-family tyrosine kinases and their signaling ligands, the Ephrins (Figure 4(c)). Binding of Ephs to Ephrins on neighboring cells leads to receptor clustering, which increases phosphotyrosine signaling in both cells. Eph-Ephrin signaling typically acts as a repulsive cue that defines cellular boundaries between tissues and organs. A recent systems-level study by Jorgensen *et al.* examined the signaling activated bidirectionally by EphB2 and Ephrin-B1 engagement.¹⁸⁷ By differentially labeling EphB2- and Ephrin-B1-expressing cells, the authors found that tyrosine kinase signaling in the two cells is asymmetric. Tyrosine kinase signaling in EphB2 cells emanated directly from EphB2 and from the nonreceptor tyrosine kinase ABL1. Conversely, in Ephrin-B1-expressing cells, the major tyrosine kinase that became activated was the insulin-like growth factor receptor, IGF1R. Eph-Ephrin-mediated segregation and epithelialization have been described as a tumor suppressor in colorectal cancers.¹⁸⁸ The repulsive interactions between EphB-positive colorectal tumor cells and surrounding EphrinB-positive normal cells leads to segregation of the transformed cells within the tumor.¹⁸⁹ This segregation may act as a tumor suppressive mechanism that inhibits cancer invasion.

Substrate stiffness and cortical tension

The tissue organization of cells is governed not only by chemical signaling networks but also by the mechanical properties of the surrounding environment. For example, breast epithelial cells undergo ductal morphogenesis when cultured in a soft low-density matrix but

not when the same matrix is crosslinked and under higher tension.¹⁹⁰ Chemically equivalent ECMs promote different cytoskeletal organization depending on the ECM rigidity.¹⁹¹ ECM tension is detected by a filamin- β 1-integrin complex in mammary epithelial cells.¹⁹⁰ Substrate stiffness provides the initial trigger for a cascade of mechanotransduction processes that influence cell contractility, migration, and cell-fate decisions. Adhesion of integrins leads to contraction of the cytoskeleton through myosin II, and the resulting actomyosin contractile force deforms either the adhesion complex (if the substrate is rigid) or the substrate (if the substrate is soft). Stiff ECM clusters integrins more effectively, which activates proliferation via ERK and cell contractility via Rho and ROCK to disrupt normal morphogenesis of breast epithelia.¹⁹² Increased ECM tension is particularly relevant for tumorigenesis. For example, collagen crosslinking is enhanced in mouse models of breast cancer through upregulation of lysyl oxidase, which promotes malignancy.¹⁹³ A major challenge lies in understanding the interplay between ECM stiffness and ECM remodeling.

Epithelial heterogeneity in morphogenesis

The textbook cytoarchitecture of many epithelial tissues is reasonably simple. However, the grossly similar appearance of epithelial cells in a tissue belies a widespread heterogeneity that is observed at the molecular level in single cells. For example, among a large pool of proliferating cells in an expanding mammary ductal tree, only a small percentage of cells have the capacity to generate ductal outgrowths containing the luminal and myoepithelial lineages that comprise normal tissue (Figure 5(a)).^{194, 195}

Aside from multilineage progenitors, luminal-restricted and myoepithelial-restricted progenitors have also been identified in breast tissue. Luminal-restricted progenitors express a hybrid set of markers between tissue stem cells and differentiated luminal epithelia.¹⁹⁶ For instance, luminal progenitor cells express luminal cytokeratin 8/18 but also cytokeratin 5/6, a basal cytokeratin that marks cells with *in vivo* repopulating capacity.¹⁹⁷ Commitment to the luminal epithelial lineage is enforced by transcription factors, such as GATA3 and FOXA1, which drive expression of hormone receptors and luminal mucins.^{196, 198} Thus, a tissue made up of two phenotypically distinct cell types (luminal and myoepithelial cells) requires at least five molecular subtypes to establish and maintain it (Figure 5(a)). Many other intermediate cell types have been described, and more await to be identified.¹⁹⁹

Molecular heterogeneity also exceeds phenotypic heterogeneity in the intestinal epithelium (Figure 5(b)). The small intestinal epithelium is organized as a cell monolayer, which bends to form crypts and villi. Rapid self-renewal takes place in crypts, whereas terminal differentiation occurs in villi. The cytoarchitecture of the intestine is more complex than the breast, in that there are four distinct lineages (enterocytes, goblet cells, enteroendocrine cells, and paneth cells). However, as in the breast, there exist many intermediate molecular states before lineage commitment.²⁰⁰ Cells expressing the leucine-rich repeat-containing G-protein-coupled receptor Lgr5 together with CD133 act as the long-lived, multipotent stem cells that reside at the crypt base to generate all cell types of the small intestine.^{201, 202} Single Lgr5-expressing cells cultured *in vitro* form intestinal organoids containing all four cell lineages.²⁰³ A second, more-quiet population of intestinal stem cells resides higher in the crypt and is marked by expression of Bmi1.²⁰⁴ Snippert *et al.* and Lopez-Garcia *et al.*, using independent labeling methods, uncovered that most intestinal stem cells divide symmetrically each day and stochastically adopt stem or transit-amplifying fates.^{205, 206} The rates at which stem cells divide and differentiated cells move to the villus are almost the same, thereby maintaining intestinal-crypt homeostasis.

In breast, intestine, and other tissues, the main limitation in discovering heterogeneities important for lineage progression is that cells in different molecular “states” are often not

microscopically distinguishable. To address this challenge, we recently developed a method for globally identifying genes with co-regulated, heterogeneous expression in cell populations.²⁰⁷ In a 3D culture model of a clonal MCF10A breast-epithelial cell line, 10–15% of all genes were found to be highly non-uniform during morphogenesis. Although explicit cell differentiation is not thought to occur in this *in vitro* model, the profiling approach could in principle be applied to epithelial development in situations where key intermediate cell states have not been defined at the molecular level.

Disruption of epithelial morphology in carcinoma

Cellular input-output relationships are substantially perturbed during epithelial tumorigenesis. Consequently, this leads to profound morphological changes at the single-cell and tissue levels. Since the discovery of oncogenes and tumor suppressors, the field of cancer biology has largely adopted a gene-centric approach to the disease.^{208, 209} However, there are multiple classic examples where morphology and microenvironment trump genetics in promoting or suppressing tumorigenesis.^{210–212} Indeed, half of the hallmarks and enabling characteristics of cancer^{213, 214} are associated with clear misregulation of the core processes described in this Review (Table 2). We discuss a few salient examples below that illustrate how epithelial cancers evolve to occupy two tissue locations—the acinar lumen and the surrounding stroma—that epithelial cells do not.

Single cells

Normal epithelial cells in tissues show a cobblestone morphology and apicobasal polarity that are stably maintained by extensive cell-cell contacts and adhesion to basement membrane (Figure 1).²¹⁵ However, during development, epithelial cells will convert to a mesenchymal phenotype in situations where dramatic cellular rearrangements are required.¹⁷⁴ Mesenchymal cells are characterized by their spindle-shaped morphology, lack of cell-cell contacts, and migration through the basement membrane. Some have argued that EMT is important for epithelial tumor progression, because transformed cells must escape the constraints of the ECM and their neighbors to invade the surrounding tissue (Table 2).

Hogan *et al.* recently examined the fate of single epithelial cells undergoing the early transformation steps that can result in an EMT.^{216, 217} Using a tetracycline-inducible Ras in MDCK cells, the authors found that single Ras transformants were mostly extruded apically from a monolayer of normal epithelial cells. Apical extrusion was promoted by Cdc42 and ROCK in Ras-transformed cells and by E-cadherin in adjacent normal cells. Disrupting either of these pathways caused Ras-transformed cells to invade the basolateral side of the epithelial monolayer in a manner reminiscent of early invasive tumors. This work suggests that normal epithelial tissues may possess tumor-suppressive mechanisms that are not cell autonomous.

Epithelial tissues

Epithelial tumors disrupt the sheet-like morphology of normal epithelia (Figure 1, 2) by occupying the tissue lumen or by invading the basement membrane. Clearance of centrally located cells, which do not have matrix contact, ordinarily involves explicit cell-death mechanisms, such as anoikis.⁵¹ However, most carcinomas display anoikis resistance and are anchorage-independent for growth and survival (Table 2). Activation of ErbB2 in 3D breast epithelial acini induces anoikis resistance⁵³ and leads to an increase in proliferation and luminal filling.²¹⁸ ErbB2 and other proliferation-inducing oncoproteins inhibit Bcl-2-family members (Bim, Bmf) that normally promote anoikis.^{34, 53, 54} In addition to preventing anoikis, ErbB2 can rescue matrix-detached cells from metabolic defects, which are associated with autophagy and luminal clearance.²¹⁹ The intersection of metabolism and

cancer has been reinvigorated over the past several years.²²⁰ By comparison, metabolic regulation during morphogenesis has been largely unexplored.

The process of invasion involves multiple steps requiring tumor cells to alter their cell-cell and cell-ECM adhesions (Table 2). Focal adhesion kinase (FAK) is recruited at an early stage to focal adhesions and acts as a pleiotropic signaling platform for cell adhesion, motility, and anchorage-independent growth. Thus, misregulated FAK activity has been implicated in tumor invasion and metastasis. FAK mRNA and protein are significantly elevated in invasive and metastatic breast tumor specimens,²²¹ and FAK is essential for oncogenic transformation and invasion induced by ErbB2-ErbB3 receptor signaling in various breast carcinoma lines.²²² One mechanism by which FAK promotes cancer invasiveness is by upregulating pericellular proteolysis of the ECM. FAK increases expression of MMP2 and MMP9 through Rac1 and c-jun N-terminal kinase activation²²³ and decreases endocytosis of MT1-MMP.²²⁴ FAK also cooperates with the nonreceptor tyrosine kinase Src in KM12C colon cancer cells to disrupt E-cadherin within adherens junctions.²²⁵ E-cadherin downregulation by FAK and Src is an important event for other invasion promoters, such as TGF β .²²⁶ FAK inhibitors are actively being explored as anticancer therapeutics.²²⁷ FAK catalytic activity is required for fibroblast motility but not proliferation,²²⁸ suggesting that such inhibitors may synergize with conventional therapies if the same mechanism holds for epithelia.

Culture models of epithelial morphogenesis

Existing systems-biology approaches are most effective when cells can be easily observed and perturbed at the molecular level.¹⁷⁰ One indication that morphogenesis is well suited for such approaches is the wealth of culture models available for exploring how epithelial tissues are shaped. As with any model, these cultures are imperfect, reflecting only how epithelial cells can organize and not necessarily how they form tissues *in vivo*. Nevertheless, they represent an important transition point for bridging systems biology and systems physiology.²²⁹

In vitro models

The form and function of all epithelial tissues are defined in three dimensions.²³⁰ However, standard *in vitro* culture creates an artificial 2D monolayer of cells. To provide a more natural epithelial context, 3D *in vitro* cultures were developed.

Most 3D culture protocols call for matrigel as the primary extracellular support.²³¹ Matrigel is prepared from the stroma-rich Engelbreth-Holm-Swarm tumor grown in the mouse peritoneum. Matrigel provides cytokines (esp. TGF β -family) and basement membrane proteins (esp. laminin, collagen IV, heparin sulfate) that are distinct from the ECM proteins that preferentially adsorb to tissue culture plastic. Matrigel thus engages a unique set of integrins and signaling pathways that promote organotypic growth. Matrigel also acts as a flexible substrate upon or within which cells tend to proliferate as isotropic spheres rather than as the sheets usually observed in 2D culture.²³² For example, when cultured in 3D with matrigel, many nontransformed epithelial lines self-organize into hollow spheres comprised of a monolayer of polarized cells (cysts) that have outside basement membranes contacting matrix and inside apical surfaces facing a central lumen. Two such lines – MDCK and MCF10A – are well established and widely used in 3D to study facets of epithelial morphogenesis.

MDCK cells show properties of the kidney distal tubule and collecting duct, such as the expression of carbonic anhydrase and a characteristic pattern of ionic selectivity.²³³ Single MDCK cells rapidly polarize when suspended in matrigel, and polarized cells then

proliferate and differentiate to form a spherical cyst. MDCK cysts develop through orderly, highly stereotyped stages, making it a great model to study the intrinsic mechanisms for epithelial polarization as directed by adhesion cues. Experimental manipulation of culture media conditions combined with genetic gain/loss-of-function experiments helped to characterize the fundamental instructive roles of ECM adhesion in cell polarity.^{29, 92, 98, 234} MDCK polarity also drives lumenogenesis in a cyst.³⁵ Polarity complexes and vesicular trafficking work together to generate the apical surface and lumen *de novo*.²³⁵ MDCK polarity is tightly controlled but is not hard-wired. Exposing MDCK cysts to HGF transiently leads to partial loss of polarity en route to tubulogenesis.¹⁰⁰ Cells extend as cords, repolarize and then become mature tubules with a continuous lumen. These polarizations stages are also similar in renal tubules *in vivo*,²³⁶ making MDCK cell an useful model for studying renal cystogenesis. Though 3D MDCK cultures offer many advantages, there are drawbacks. A substantial disadvantage for studying size control is that MDCK cysts do not arrest their proliferation in 3D.²³⁷ A second inconvenience is that because of their canine origin, all RNAi-based reagents must be designed in house.²³⁸ Nonetheless, MDCK cultures remain the preferred 3D model for polarity.

Another widely used model that complements MDCK cysts is 3D culture of the immortalized human breast epithelial cell line, MCF10A.²³⁹ Seeding single MCF10A cells atop a matrigel bed and in the presence of a dilute matrigel solution promotes the formation of growth-arrested acini in ~2 weeks. The development of MCF10A acini involves the coordination of a proliferative phase in the outer cells during days 1–8 and an apoptotic phase in central cells during days 6–12.^{51, 52} The control of proliferation and death-vs.-survival are processes that are well suited to this model and may reflect facets of the regulation *in vivo*.³⁴ Moreover, as a nontumorigenic cell line, MCF10A cells can be used to study the effects of oncogenes and tumor suppressor genes on morphogenesis. These manipulations can draw from the wealth of resources available for human genes.^{240, 241} Yet, the MCF10A model cannot compete with the strengths of MDCK cysts. Compared with MDCK cells, which begin polarization and lumen formation during the first cell division,²⁴² MCF10A acini are slow to develop. In addition, the overall polarity of MCF10A is much less conclusive when compared with MDCK cells. The Golgi apparatus orients apically and ECM is deposited basolaterally in MCF10A acini, but other important polarity complexes do not appear to form endogenously.^{9, 218, 243} A specific disadvantage of the MCF10A model for *in vivo* relevance is that by using only one cell type, it omits the dual luminal-myoeepithelial layers that comprise actual breast tissue (Figure 5(a)). To track cell behavior in mixed cell types, other techniques are needed to provide insight into multilayered epithelia.

Ex vivo models

The architecture of normal epithelium *in vivo* is defined by epithelial interactions with both the ECM and the stromal mesenchyme. Thus, traditional 2D and even 3D cell cultures incompletely describe epithelial morphogenesis by using a single cell type. *Ex vivo* cultures of tissue progenitor or entire epithelial organs seek to address this deficiency in a format that is easily observable.

Ex vivo cultures can start with entire epithelial organs, tissue fragments, or just single cells. For example, whole-corneal organ cultures using an air-interface technique have been used for evaluating ocular irritation.^{244–246} The major drawback of organ culture is an inability to perfuse large tissues adequately, causing a progressive decline in function during the course of an experiment.²⁴⁷ More recently, smaller epithelial fragments isolated from mouse mammary glands were used to study epithelial cell reorganization, collective migration, and ductal elongation.⁵⁷ Fragment cultures strike a compromise by maintaining the microenvironment locally in a format that is more amenable to *in vitro* conditions. When

multipotent progenitors can be reliably identified, *ex vivo* morphogenesis cultures of single primary cells may be possible. For example, single stem cells sorted from crypts of small intestine can rebuild entire crypt–villus units during long-term 3D culture.²⁰³ Single-cell cultures lack tissue stroma and must recreate an appropriate ECM, but they can be tremendously powerful for tracking differentiation in epithelial tissues.²⁴⁸

The major advantage of all *ex vivo* formats is that they are compatible with long-term time-lapse imaging techniques. Combining fragment cultures and confocal microscopy revealed a sequence of steps underlying mammary branching morphogenesis.⁵⁷ *Ex vivo* models also enable reliable identification and tracking of single-cell behavior in mixed cell lineages.²⁰³ Last, *ex vivo* cultures provide control of the local cellular microenvironment in space and time.⁵⁰ Advances in micropatterning and their application to morphogenesis will allow researchers to explore non-native microenvironments and their impact on tissue structure.^{249, 250}

Computational models of epithelial morphogenesis

An indispensable component of systems-biology research is the development and use of computational models. Many recent reviews in this journal have covered modeling from the perspective of epithelial biology and cancer biology.^{251–253} In this section, we very briefly review the major relevant classes of models and their recent application to questions in morphogenesis and tumorigenesis.

Continuous modeling

Continuum models describe spatial tissue parameters as continuous fields that vary in time. Capturing the dual time-space dependence mathematically requires partial differential equations, which typically must be solved numerically. Nonetheless, there is a long history in applying continuous models to morphogenesis, dating back to the proposal of the morphogen theory itself.²⁵⁴ Within continuum models, parameters such as tissue or tumor growth rate or oxygen diffusion can be directly estimated through experiments, because they correspond to macroscopic tissue or tumor properties (Table 3). For example, a continuum model was used to examine the effects of proliferation-induced mechanical stresses and hypoxic stresses on production of proteolytic enzymes during progression of ductal carcinoma in situ (DCIS) of the breast.²⁵⁵ This model described how mechanical effects dominate in stimulating the protease production that alters the mechanical properties of the ductal walls and predicted the region that tumor cells are most likely to invade into the surrounding tissue.

Cell sheets are good tissues to study with continuum models.²⁵⁶ For example, an advancing enterocyte monolayer was modeled as a 1D continuum of springs to mimic physical tension between cells.²⁵⁷ A 2D continuum approach was adopted to model the colorectal crypt epithelium as a growing beam attached to an underlying lamina.²⁵⁸ Springs—buckled by the combination of cell proliferation, movement, and cellular attachment—act to model the process of crypt budding and fission. Continuum models encompass not only cell-tissue mechanics but also transport processes. Cell sheet restoration during wound repair is compatible with a reaction-diffusion model, Fisher's equation, originally used to model population spread.^{259, 260} This model was further elaborated to connect receptor activation and intracellular signaling to proliferation and migration responses during 2D wound healing.²⁶¹ In this way, continuum models provide a straightforward way to combine molecular and biophysical processes within a common quantitative framework.

Discrete modeling

Classic implementations of continuum models do not capture the different properties and behaviors of individual cells, which is an important facet of epithelial tissues (Table 3). One can track the autonomous behavior of single cells by discrete models based on rules describing key biological processes that depend on the microenvironment. Discrete models of morphogenesis are consistent with the historical positional-information theory of tissue patterning.²⁶² In contrast to continuum model parameters, which can often be directly estimated from experiments, the rules of discrete models must first be defined heuristically. However, rule sets are biologically inspired and their single-cell predictions can be directly compared with experimental observations during model testing and refinement.

Several models have captured the organotypic phenotypes of in vitro cell cultures, which mimic facets of epithelial tissues. Kim et al. created an in silico study of human alveolar type II (AT II) 3D cell cultures, which revealed a set of cell operating rules that are sufficient for describing the development of cyst-like structures.²⁶³ This study was further refined to explore the underlying mechanism of AT II wound healing.²⁶⁴ Grant *et al.* also built a discrete model of 3D MDCK cysts.²⁶⁵ The authors revised their initial rule set based on the model's ability to capture epithelial morphogenesis under four growth conditions that give rise to different multicellular arrangements. Interestingly, the key modification to this model was the addition of an explicit polarized cell state, whose stability depended on the local cellular and ECM environment. Thus, only a few experimental observations may be required to constrain discrete-model rule sets, provided that these constraints are stringent.

Discrete models are also easily revised to apply to different biological circumstances. For example, modeling the response of epithelial cells to contact inhibition has been used to wound healing in monolayer cell cultures.²⁶⁶ This study was extended to explore the effects of normal cell-cell interactions on the dynamics of population growth.²⁶⁷ The model predicted non-cell-autonomous effects on population dynamics and single-cell behavior when individual cells lose E-cadherin expression. Rejniak developed a single-cell-based model called *IBCell* (Immersed Boundary model of a Cell) to model the evolution of solid tumors.²⁶⁸ The model framework was subsequently adjusted to different applications, such as to represent the cross-section of normally growing epithelial acini and to study acinar development and stability.^{269, 270} These studies show that local interactions between individual cells and their microenvironment guide the dynamic development of epithelial acini (Figure 2(c)). More recently, *IBCell* was tuned to experimental data from 3D culture of MCF10A cells.^{239, 271} This customized model captures the abnormal acinar morphology observed when cells express an oncogenic HER2-YVMA mutant.^{271, 272} In the future, it will be interesting to explore how many cancer-related morphogenetic abnormalities can be captured under a common discrete rule set.

Hybrid modeling

Discrete models thrive at single-cell simulations but struggle to handle continuous variables, such as diffusible growth factors and oxygen tension (Table 3). To address this, so-called hybrid models attempt to integrate continuum and discrete descriptions linking cell behavior with macroscopic tissue properties. Diffusible factors are described as continuum fields, with cells modeled as individual discrete elements that evolve dynamically in response to their local conditions. Hybrid models can be built from scratch or can result from the fusion of pre-existing continuum and discrete models. For example, a hybrid model of tumor growth was built from earlier continuum and discrete models to account for intratumor processes, host-tumor interactions, and cell-level heterogeneity.²⁷³

Computational resources have been developed to allow the integration of molecular, cell, and tissue scales in a hybrid framework. For example, Cickovski *et al.* have built a computational package, *CompuCell3D*, designed for hybrid modeling of morphogenesis.²⁷⁴ Another hybrid platform, *Epitheliome*, was developed to model epithelial tissue specifically and has been used to examine the effects of growth factors on epithelial development.²⁷⁵ Hybrid models can provide the most-realistic coupling of biophysical processes at the expense of a higher number of parameters that must be estimated at an increased computational cost.

One active research direction in the area of integrative cancer biology involves the development of hybrid models for tumor invasion. An early hybrid model was originally developed by Anderson to describe solid tumor growth based on both continuous (ECM, matrix-degrading enzymes and oxygen) and discrete (tumor cells) factors.²⁷⁶ This model was later extended to include cell-cell adhesion to evaluate how individual cells and cell-matrix interactions may affect tumor morphology.²⁷⁷ Anderson's follow-up studies quantitatively characterized tumor invasion as a function of microenvironmental selective factors,²⁷⁸ linking low nutrient availability with a fingered tumor morphology.²⁷⁹ These studies correlate with another model, which considered individual cells interacting with surrounding nutrients and showed that tumor microenvironments cause invasive projections from solid tumors.²⁸⁰ Anderson and coworkers further showed by hybrid modeling that tumorigenic cells were selected under constrained growth conditions with limited nutrients.²⁸¹ Aggressive phenotypes that produce tumor fingering are favored in poor, but not rich, nutrient microenvironments.²⁸² Another hybrid model recently added a mechanical component to the finger-like tumor morphology, arising from the combined strength of cell-cell adhesion and haptotaxis.²⁸³ These models converge on a common prediction that transport limitations of diffusible factors leads to tissue instability, creating a platform for tumor evolution and invasion.

Conclusion and Future Directions

To simplify a daunting challenge, our Advanced Review has sought to break down epithelial morphogenesis into tractable elements. Many of these elements have been modeled computationally, measured quantitatively, or both, and we expect that many more will be soon. Conversely, very few of the published computational models that we discussed were explicitly focused on epithelial morphogenesis. This does not make the current models irrelevant, because it should be possible to co-opt them for morphogenesis-specific applications. For example, models of diffusible factors have been shown to make accurate predictions across different epithelial lineages and genetic backgrounds.²⁸⁴⁻²⁸⁶ Defining the individual elements, characterizing their biochemical properties, and modeling their emergent systems-level properties is thus an important first step.

It remains unclear whether such elements can be treated as true “modules”, which can be studied in isolation and then wired together afterwards.² For clarity, we have avoided the topic of crosstalk between elements, but this undoubtedly occurs, and reports in the literature are too numerous to mention. One major obstacle to a more-integrative understanding is that we lack general, effective, and efficient approaches for incorporating crosstalk without getting overwhelmed by it. This hurdle will not be overcome without adopting an interdisciplinary systems approach.

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References

1. Ideker T, Galitski T, Hood L. A new approach to decoding life: systems biology. *Annu Rev Genomics Hum Genet.* 2001; 2:343–372. [PubMed: 11701654]
2. Hartwell LH, Hopfield JJ, Leibler S, Murray AW. From molecular to modular cell biology. *Nature.* 1999; 402:C47–52. [PubMed: 10591225]
3. Cereijido M, Contreras RG, Shoshani L. Cell adhesion, polarity, and epithelia in the dawn of metazoans. *Physiol Rev.* 2004; 84:1229–1262. [PubMed: 15383651]
4. Shin K, Fogg VC, Margolis B. Tight junctions and cell polarity. *Annu Rev Cell Dev Biol.* 2006; 22:207–235. [PubMed: 16771626]
5. Tepass U, Tanentzapf G, Ward R, Fehon R. Epithelial cell polarity and cell junctions in *Drosophila*. *Annu Rev Genet.* 2001; 35:747–784. [PubMed: 11700298]
6. Humbert PO, Dow LE, Russell SM. The Scribble and Par complexes in polarity and migration: friends or foes? *Trends Cell Biol.* 2006; 16:622–630. [PubMed: 17067797]
7. Itoh M, Nagafuchi A, Yonemura S, Kitani-Yasuda T, Tsukita S. The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. *J Cell Biol.* 1993; 121:491–502. [PubMed: 8486731]
8. St Johnston D, Ahringer J. Cell polarity in eggs and epithelia: parallels and diversity. *Cell.* 2010; 141:757–774. [PubMed: 20510924]
9. Zhan L, Rosenberg A, Bergami KC, Yu M, Xuan Z, Jaffe AB, Allred C, Muthuswamy SK. Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma. *Cell.* 2008; 135:865–878. [PubMed: 19041750]
10. McCaffrey LM, Macara IG. The Par3/aPKC interaction is essential for end bud remodeling and progenitor differentiation during mammary gland morphogenesis. *Genes Dev.* 2009; 23:1450–1460. [PubMed: 19528321]
11. Moreno-Bueno G, Portillo F, Cano A. Transcriptional regulation of cell polarity in EMT and cancer. *Oncogene.* 2008; 27:6958–6969. [PubMed: 19029937]
12. Devenport D, Fuchs E. Planar polarization in embryonic epidermis orchestrates global asymmetric morphogenesis of hair follicles. *Nat Cell Biol.* 2008; 10:1257–1268. [PubMed: 18849982]
13. Wu J, Mlodzik M. A quest for the mechanism regulating global planar cell polarity of tissues. *Trends Cell Biol.* 2009; 19:295–305. [PubMed: 19560358]
14. Amonlirdviman K, Khare NA, Tree DR, Chen WS, Axelrod JD, Tomlin CJ. Mathematical modeling of planar cell polarity to understand domineering nonautonomy. *Science.* 2005; 307:423–426. [PubMed: 15662015]
15. Lawrence PA, Struhl G, Casal J. Planar cell polarity: one or two pathways? *Nat Rev Genet.* 2007; 8:555–563. [PubMed: 17563758]
16. Martin AC, Kaschube M, Wieschaus EF. Pulsed contractions of an actin-myosin network drive apical constriction. *Nature.* 2009; 457:495–499. [PubMed: 19029882]
17. Lecuit T, Lenne PF. Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nat Rev Mol Cell Biol.* 2007; 8:633–644. [PubMed: 17643125]
18. Hickson GR, Echard A, O'Farrell PH. Rho-kinase controls cell shape changes during cytokinesis. *Curr Biol.* 2006; 16:359–370. [PubMed: 16488869]
19. Ivanov AI, Hopkins AM, Brown GT, Gerner-Smidt K, Babbitt BA, Parkos CA, Nusrat A. Myosin II regulates the shape of three-dimensional intestinal epithelial cysts. *J Cell Sci.* 2008; 121:1803–1814. [PubMed: 18460584]
20. Gibson MC, Patel AB, Nagpal R, Perrimon N. The emergence of geometric order in proliferating metazoan epithelia. *Nature.* 2006; 442:1038–1041. [PubMed: 16900102]

21. Farhadifar R, Roper JC, Aigouy B, Eaton S, Julicher F. The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. *Curr Biol*. 2007; 17:2095–2104. [PubMed: 18082406]
22. Rauzi M, Verant P, Lecuit T, Lenne PF. Nature and anisotropy of cortical forces orienting *Drosophila* tissue morphogenesis. *Nat Cell Biol*. 2008; 10:1401–1410. [PubMed: 18978783]
23. Sawyer JM, Harrell JR, Shemer G, Sullivan-Brown J, Roh-Johnson M, Goldstein B. Apical constriction: a cell shape change that can drive morphogenesis. *Dev Biol*. 2010; 341:5–19. [PubMed: 19751720]
24. Bryant DM, Mostov KE. From cells to organs: building polarized tissue. *Nature Reviews Molecular Cell Biology*. 2008; 9:887–901.
25. Andrew DJ, Ewald AJ. Morphogenesis of epithelial tubes: Insights into tube formation, elongation, and elaboration. *Developmental Biology*. 2010; 341:34–55. [PubMed: 19778532]
26. Lubarsky B, Krasnow MA. Tube morphogenesis: Making and shaping biological tubes. *Cell*. 2003; 112:19–28. [PubMed: 12526790]
27. Rasmussen JP, English K, Tenlen JR, Priess JR. Notch signaling and morphogenesis of single-cell tubes in the *C. elegans* digestive tract. *Dev Cell*. 2008; 14:559–569. [PubMed: 18410731]
28. Ghabrial AS, Krasnow MA. Social interactions among epithelial cells during tracheal branching morphogenesis. *Nature*. 2006; 441:746–749. [PubMed: 16760977]
29. Yu W, Datta A, Leroy P, O'Brien LE, Mak G, Jou TS, Matlin KS, Mostov KE, Zegers MM. Beta1-integrin orients epithelial polarity via Rac1 and laminin. *Mol Biol Cell*. 2005; 16:433–445. [PubMed: 15574881]
30. Tawk M, Araya C, Lyons DA, Reugels AM, Girdler GC, Bayley PR, Hyde DR, Tada M, Clarke JDW. A mirror-symmetric cell division that orchestrates neuroepithelial morphogenesis. *Nature*. 2007; 446:797–800. [PubMed: 17392791]
31. Jaffe AB, Kaji N, Durgan J, Hall A. Cdc42 controls spindle orientation to position the apical surface during epithelial morphogenesis. *Journal of Cell Biology*. 2008; 183:625–633. [PubMed: 19001128]
32. Bryant DM, Datta A, Rodriguez-Fraticelli AE, Peranen J, Martin-Belmonte F, Mostov KE. A molecular network for de novo generation of the apical surface and lumen. *Nature Cell Biology*. 2010; 12:1035–U1024.
33. Melnick M, Jaskoll T. Mouse submandibular gland morphogenesis: a paradigm for embryonic signal processing. *Crit Rev Oral Biol Med*. 2000; 11:199–215. [PubMed: 12002815]
34. Maillieux AA, Overholtzer M, Schmelzle T, Bouillet P, Strasser A, Brugge JS. BIM regulates apoptosis during mammary ductal morphogenesis, and its absence reveals alternative cell death mechanisms. *Dev Cell*. 2007; 12:221–234. [PubMed: 17276340]
35. Martin-Belmonte F, Yu W, Rodriguez-Fraticelli AE, Ewald AJ, Werb Z, Alonso MA, Mostov K. Cell-polarity dynamics controls the mechanism of lumen formation in epithelial morphogenesis. *Curr Biol*. 2008; 18:507–513. [PubMed: 18394894]
36. Bagnat M, Cheung ID, Mostov KE, Stainier D.Y.R. Genetic control of single lumen formation in the zebrafish gut. *Nature Cell Biology*. 2007; 9:954–U119.
37. Kesavan G, Sand FW, Greiner TU, Johansson JK, Kobberup S, Wu X, Brakebusch C, Semb H. Cdc42-mediated tubulogenesis controls cell specification. *Cell*. 2009; 139:791–801. [PubMed: 19914171]
38. Samakovlis C, Manning G, Steneberg P, Hacohen N, Cantera R, Krasnow MA. Genetic control of epithelial tube fusion during *Drosophila* tracheal development. *Development*. 1996; 122:3531–3536. [PubMed: 8951068]
39. Nakanishi Y, Morita T, Nogawa H. Cell proliferation is not required for the initiation of early cleft formation in mouse embryonic submandibular epithelium in vitro. *Development*. 1987; 99:429–437. [PubMed: 3115749]
40. Oblander SA, Zhou Z, Galvez BG, Starcher B, Shannon JM, Durbeek M, Arroyo AG, Tryggvason K, Apte SS. Distinctive functions of membrane type 1 matrix-metalloprotease (MT1-MMP or MMP-14) in lung and submandibular gland development are independent of its role in pro-MMP-2 activation. *Dev Biol*. 2005; 277:255–269. [PubMed: 15572153]

41. Ewald AJ, Brenot A, Duong M, Chan BS, Werb Z. Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. *Developmental Cell*. 2008; 14:570–581. [PubMed: 18410732]
42. Moore KA, Polte T, Huang S, Shi B, Alsberg E, Sunday ME, Ingber DE. Control of basement membrane remodeling and epithelial branching morphogenesis in embryonic lung by Rho and cytoskeletal tension. *Developmental Dynamics*. 2005; 232:268–281. [PubMed: 15614768]
43. Gilles C, Polette M, Coraux C, Tournier JM, Meneguzzi G, Munaut C, Volders L, Rousselle P, Birembaut P, Foidart JM. Contribution of MT1-MMP and of human laminin-5 gamma2 chain degradation to mammary epithelial cell migration. *J Cell Sci*. 2001; 114:2967–2976. [PubMed: 11686300]
44. Friedl P, Wolf K. Tube travel: the role of proteases in individual and collective cancer cell invasion. *Cancer Res*. 2008; 68:7247–7249. [PubMed: 18794108]
45. Gudjonsson T, Ronnov-Jessen L, Villadsen R, Rank F, Bissell MJ, Petersen OW. Normal and tumor-derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. *Journal of Cell Science*. 2002; 115:39–50. [PubMed: 11801722]
46. Davies JA. Do different branching epithelia use a conserved developmental mechanism? *Bioessays*. 2002; 24:937–948. [PubMed: 12325126]
47. Sternlicht MD, Sunnarborg SW, Kourou-Mehr H, Yu Y, Lee DC, Werb Z. Mammary ductal morphogenesis requires paracrine activation of stromal EGFR via ADAM17-dependent shedding of epithelial amphiregulin. *Development*. 2005; 132:3923–3933. [PubMed: 16079154]
48. Lu PF, Werb Z. Patterning Mechanisms of Branched Organs. *Science*. 2008; 322:1506–1509. [PubMed: 19056977]
49. Ewan KB, Shyamala G, Ravani SA, Tang Y, Akhurst R, Wakefield L, Barcellos-Hoff MH. Latent transforming growth factor-beta activation in mammary gland - Regulation by ovarian hormones affects ductal and alveolar proliferation. *American Journal of Pathology*. 2002; 160:2081–2093. [PubMed: 12057913]
50. Nelson CM, VanDuijn MM, Inman JL, Fletcher DA, Bissell MJ. Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures. *Science*. 2006; 314:298–300. [PubMed: 17038622]
51. Debnath J, Mills KR, Collins NL, Reginato MJ, Muthuswamy SK, Brugge JS. The role of apoptosis in creating and maintaining luminal space within normal and oncogene-expressing mammary acini. *Cell*. 2002; 111:29–40. [PubMed: 12372298]
52. Debnath J, Brugge JS. Modelling glandular epithelial cancers in three-dimensional cultures. *Nat Rev Cancer*. 2005; 5:675–688. [PubMed: 16148884]
53. Reginato MJ, Mills KR, Paulus JK, Lynch DK, Sgroi DC, Debnath J, Muthuswamy SK, Brugge JS. Integrins and EGFR coordinately regulate the pro-apoptotic protein Bim to prevent anoikis. *Nat Cell Biol*. 2003; 5:733–740. [PubMed: 12844146]
54. Reginato MJ, Mills KR, Becker EB, Lynch DK, Bonni A, Muthuswamy SK, Brugge JS. Bim regulation of lumen formation in cultured mammary epithelial acini is targeted by oncogenes. *Mol Cell Biol*. 2005; 25:4591–4601. [PubMed: 15899862]
55. Schmelzle T, Mailleux AA, Overholtzer M, Carroll JS, Solimini NL, Lightcap ES, Veiby OP, Brugge JS. Functional role and oncogene-regulated expression of the BH3-only factor Bim in mammary epithelial anoikis and morphogenesis. *Proc Natl Acad Sci U S A*. 2007; 104:3787–3792. [PubMed: 17360431]
56. Mills KR, Reginato M, Debnath J, Queenan B, Brugge JS. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is required for induction of autophagy during lumen formation in vitro. *Proc Natl Acad Sci U S A*. 2004; 101:3438–3443. [PubMed: 14993595]
57. Ewald AJ, Brenot A, Duong M, Chan BS, Werb Z. Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. *Dev Cell*. 2008; 14:570–581. [PubMed: 18410732]
58. Fata JE, Mori H, Ewald AJ, Zhang H, Yao E, Werb Z, Bissell MJ. The MAPK(ERK-1,2) pathway integrates distinct and antagonistic signals from TGFalpha and FGF7 in morphogenesis of mouse mammary epithelium. *Dev Biol*. 2007; 306:193–207. [PubMed: 17448457]

59. Yerbes R, Palacios C, Reginato MJ, Lopez-Rivas A. Cellular FLIP(L) plays a survival role and regulates morphogenesis in breast epithelial cells. *Biochim Biophys Acta*. 2010;1010.1016/j.bbamer.2010.1010.1003
60. Albeck JG, Burke JM, Spencer SL, Lauffenburger DA, Sorger PK. Modeling a snap-action, variable-delay switch controlling extrinsic cell death. *PLoS Biol*. 2008; 6:2831–2852. [PubMed: 19053173]
61. Spencer SL, Gaudet S, Albeck JG, Burke JM, Sorger PK. Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis. *Nature*. 2009; 459:428–432. [PubMed: 19363473]
62. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. *Nat Rev Mol Cell Biol*. 2007; 8:221–233. [PubMed: 17318226]
63. Rowe RG, Weiss SJ. Navigating ECM barriers at the invasive front: the cancer cell-stroma interface. *Annu Rev Cell Dev Biol*. 2009; 25:567–595. [PubMed: 19575644]
64. Rebutini IT, Myers C, Lassiter KS, Surmak A, Szabova L, Holmbeck K, Pedchenko V, Hudson BG, Hoffman MP. MT2-MMP-dependent release of collagen IV NC1 domains regulates submandibular gland branching morphogenesis. *Dev Cell*. 2009; 17:482–493. [PubMed: 19853562]
65. Apte SS. A disintegrin-like and metalloprotease (reprolysin-type) with thrombospondin type 1 motif (ADAMTS) superfamily: functions and mechanisms. *J Biol Chem*. 2009; 284:31493–31497. [PubMed: 19734141]
66. McCulloch DR, Nelson CM, Dixon LJ, Silver DL, Wylie JD, Lindner V, Sasaki T, Cooley MA, Argraves WS, Apte SS. ADAMTS metalloproteases generate active versican fragments that regulate interdigital web regression. *Dev Cell*. 2009; 17:687–698. [PubMed: 19922873]
67. Gudjonsson T, Ronnov-Jessen L, Villadsen R, Rank F, Bissell MJ, Petersen OW. Normal and tumor-derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. *J Cell Sci*. 2002; 115:39–50. [PubMed: 11801722]
68. Streuli CH. Maspin is a tumour suppressor that inhibits breast cancer tumour metastasis in vivo. *Breast Cancer Res*. 2002; 4:137–140. [PubMed: 12100737]
69. Lauffenburger DA, Horwitz AF. Cell migration: a physically integrated molecular process. *Cell*. 1996; 84:359–369. [PubMed: 8608589]
70. Giampieri S, Manning C, Hooper S, Jones L, Hill CS, Sahai E. Localized and reversible TGFbeta signalling switches breast cancer cells from cohesive to single cell motility. *Nat Cell Biol*. 2009; 11:1287–1296. [PubMed: 19838175]
71. Shakya R, Watanabe T, Costantini F. The role of GDNF/Ret signaling in ureteric bud cell fate and branching morphogenesis. *Dev Cell*. 2005; 8:65–74. [PubMed: 15621530]
72. Larsen M, Wei C, Yamada KM. Cell and fibronectin dynamics during branching morphogenesis. *J Cell Sci*. 2006; 119:3376–3384. [PubMed: 16882689]
73. Batlle E, Henderson JT, Beghtel H, van den Born MM, Sancho E, Huls G, Meeldijk J, Robertson J, van de Wetering M, Pawson T, et al. Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. *Cell*. 2002; 111:251–263. [PubMed: 12408869]
74. Pearson GW, Hunter T. Real-time imaging reveals that noninvasive mammary epithelial acini can contain motile cells. *J Cell Biol*. 2007; 179:1555–1567. [PubMed: 18166657]
75. Chi X, Michos O, Shakya R, Riccio P, Enomoto H, Licht JD, Asai N, Takahashi M, Ohgami N, Kato M, et al. Ret-dependent cell rearrangements in the Wolffian duct epithelium initiate ureteric bud morphogenesis. *Dev Cell*. 2009; 17:199–209. [PubMed: 19686681]
76. Mori H, Gjorevski N, Inman JL, Bissell MJ, Nelson CM. Self-organization of engineered epithelial tubules by differential cellular motility. *Proc Natl Acad Sci U S A*. 2009; 106:14890–14895. [PubMed: 19706461]
77. Rorth P. Collective cell migration. *Annu Rev Cell Dev Biol*. 2009; 25:407–429. [PubMed: 19575657]
78. Maddugoda MP, Crampton MS, Shewan AM, Yap AS. Myosin VI and vinculin cooperate during the morphogenesis of cadherin cell-cell contacts in mammalian epithelial cells. *Journal of Cell Biology*. 2007; 178:529–540. [PubMed: 17664339]

79. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, et al. Tensional homeostasis and the malignant phenotype. *Cancer Cell*. 2005; 8:241–254. [PubMed: 16169468]
80. Grose R, Hutter C, Bloch W, Thorey I, Watt FM, Fassler R, Brakebusch C, Werner S. A crucial role of beta 1 integrins for keratinocyte migration in vitro and during cutaneous wound repair. *Development*. 2002; 129:2303–2315. [PubMed: 11959837]
81. Zelenka PS, Arpitha P. Coordinating cell proliferation and migration in the lens and cornea. *Semin Cell Dev Biol*. 2008; 19:113–124. [PubMed: 18035561]
82. Omelchenko T, Vasiliev JM, Gelfand IM, Feder HH, Bonder EM. Rho-dependent formation of epithelial “leader” cells during wound healing. *Proc Natl Acad Sci U S A*. 2003; 100:10788–10793. [PubMed: 12960404]
83. Poujade M, Grasland-Mongrain E, Hertzog A, Jouanneau J, Chavrier P, Ladoux B, Buguin A, Silberzan P. Collective migration of an epithelial monolayer in response to a model wound. *Proc Natl Acad Sci U S A*. 2007; 104:15988–15993. [PubMed: 17905871]
84. Trepas X, Wasserman MR, Angelini TE, Millet E, Weitz DA, Butler JP, Fredberg JJ. Physical forces during collective cell migration. *Nature Physics*. 2009; 5:426–430.
85. Ladoux B. BIOPHYSICS Cells guided on their journey. *Nature Physics*. 2009; 5:377–378.
86. Haas P, Gilmour D. Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Dev Cell*. 2006; 10:673–680. [PubMed: 16678780]
87. Caussinus E, Colombelli J, Affolter M. Tip-cell migration controls stalk-cell intercalation during *Drosophila* tracheal tube elongation. *Curr Biol*. 2008; 18:1727–1734. [PubMed: 19026547]
88. Mori H, Gjorevski N, Inman JL, Bissell MJ, Nelson CM. Self-organization of engineered epithelial tubules by differential cellular motility. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:14890–14895. [PubMed: 19706461]
89. Ikeya T, Hayashi S. Interplay of Notch and FGF signaling restricts cell fate and MAPK activation in the *Drosophila* trachea. *Development*. 1999; 126:4455–4463. [PubMed: 10498681]
90. Greenhalgh DG, Sprugel KH, Murray MJ, Ross R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. *Am J Pathol*. 1990; 136:1235–1246. [PubMed: 2356856]
91. Jaffe AB, Kaji N, Durgan J, Hall A. Cdc42 controls spindle orientation to position the apical surface during epithelial morphogenesis. *J Cell Biol*. 2008; 183:625–633. [PubMed: 19001128]
92. O’Brien LE, Jou TS, Pollack AL, Zhang Q, Hansen SH, Yurchenco P, Mostov KE. Rac1 orientates epithelial apical polarity through effects on basolateral laminin assembly. *Nat Cell Biol*. 2001; 3:831–838. [PubMed: 11533663]
93. Martin-Belmonte F, Gassama A, Datta A, Yu W, Rescher U, Gerke V, Mostov K. PTEN-mediated apical segregation of phosphoinositides controls epithelial morphogenesis through Cdc42. *Cell*. 2007; 128:383–397. [PubMed: 17254974]
94. Wells CD, Fawcett JP, Traweger A, Yamanaka Y, Goudreaux M, Elder K, Kulkarni S, Gish G, Virag C, Lim C, et al. A Rho1/Amot complex regulates the Cdc42 GTPase and apical-polarity proteins in epithelial cells. *Cell*. 2006; 125:535–548. [PubMed: 16678097]
95. Qin Y, Meisen WH, Hao Y, Macara IG. Tuba, a Cdc42 GEF, is required for polarized spindle orientation during epithelial cyst formation. *J Cell Biol*. 2010; 189:661–669. [PubMed: 20479467]
96. Rodriguez-Fraticelli AE, Vergara-Jauregui S, Eastburn DJ, Datta A, Alonso MA, Mostov K, Martin-Belmonte F. The Cdc42 GEF Intersectin 2 controls mitotic spindle orientation to form the lumen during epithelial morphogenesis. *J Cell Biol*. 2010; 189:725–738. [PubMed: 20479469]
97. Nejsum LN, Nelson WJ. A molecular mechanism directly linking E-cadherin adhesion to initiation of epithelial cell surface polarity. *J Cell Biol*. 2007; 178:323–335. [PubMed: 17635938]
98. Yu W, Shewan AM, Brakeman P, Eastburn DJ, Datta A, Bryant DM, Fan QW, Weiss WA, Zegers MM, Mostov KE. Involvement of RhoA, ROCK I and myosin II in inverted orientation of epithelial polarity. *EMBO Rep*. 2008; 9:923–929. [PubMed: 18660750]
99. Adams SA, Smith ME, Cowley GP, Carr LA. Reversal of glandular polarity in the lymphovascular compartment of breast cancer. *J Clin Pathol*. 2004; 57:1114–1117. [PubMed: 15452174]
100. Zegers MM, O’Brien LE, Yu W, Datta A, Mostov KE. Epithelial polarity and tubulogenesis in vitro. *Trends Cell Biol*. 2003; 13:169–176. [PubMed: 12667754]

101. Orr AW, Helmke BP, Blackman BR, Schwartz MA. Mechanisms of mechanotransduction. *Dev Cell*. 2006; 10:11–20. [PubMed: 16399074]
102. Montesano R, Matsumoto K, Nakamura T, Orci L. Identification of a Fibroblast-Derived Epithelial Morphogen as Hepatocyte Growth-Factor. *Cell*. 1991; 67:901–908. [PubMed: 1835669]
103. Bussolino F, Drenzo MF, Ziche M, Bocchietto E, Olivero M, Naldini L, Gaudino G, Tamagnone L, Coffe A, Comoglio PM. Hepatocyte Growth-Factor Is a Potent Angiogenic Factor Which Stimulates Endothelial-Cell Motility and Growth. *Journal of Cell Biology*. 1992; 119:629–641. [PubMed: 1383237]
104. Park M, Dean M, Cooper CS, Schmidt M, O'Brien SJ, Blair DG, Vande Woude GF. Mechanism of met oncogene activation. *Cell*. 1986; 45:895–904. [PubMed: 2423252]
105. Bottaro DP, Rubin JS, Faletto DL, Chan AML, Kmieciak TE, Vandewoude GF, Aaronson SA. Identification of the Hepatocyte Growth-Factor Receptor as the C-Met Protooncogene Product. *Science*. 1991; 251:802–804. [PubMed: 1846706]
106. Naldini L, Weidner KM, Vigna E, Gaudino G, Bardelli A, Ponzetto C, Narsimhan RP, Hartmann G, Zarnegar R, Michalopoulos GK, et al. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. *EMBO J*. 1991; 10:2867–2878. [PubMed: 1655405]
107. Ponzetto C, Bardelli A, Zhen Z, Maina F, dalla Zonca P, Giordano S, Graziani A, Panayotou G, Comoglio PM. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. *Cell*. 1994; 77:261–271. [PubMed: 7513258]
108. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nature Reviews Molecular Cell Biology*. 2003; 4:915–925.
109. Weidner KM, Di Cesare S, Sachs M, Brinkmann V, Behrens J, Birchmeier W. Interaction between Gab1 and the c-Met receptor tyrosine kinase is responsible for epithelial morphogenesis. *Nature*. 1996; 384:173–176. [PubMed: 8906793]
110. Sakkab D, Lewitzky M, Posern G, Schaeper U, Sachs M, Birchmeier W, Feller SM. Signaling of hepatocyte growth factor/scatter factor (HGF) to the small GTPase Rap1 via the large docking protein Gab1 and the adapter protein CRKL. *J Biol Chem*. 2000; 275:10772–10778. [PubMed: 10753869]
111. Xiao GH, Jeffers M, Bellacosa A, Mitsuuchi Y, Vande Woude GF, Testa JR. Anti-apoptotic signaling by hepatocyte growth factor/Met via the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways. *Proc Natl Acad Sci U S A*. 2001; 98:247–252. [PubMed: 11134526]
112. Kennedy SG, Wagner AJ, Conzen SD, Jordan J, Bellacosa A, Tsichlis PN, Hay N. The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. *Genes Dev*. 1997; 11:701–713. [PubMed: 9087425]
113. Franke R, Muller M, Wundrack N, Gilles ED, Klamt S, Kahne T, Naumann M. Host-pathogen systems biology: logical modelling of hepatocyte growth factor and *Helicobacter pylori* induced c-Met signal transduction. *BMC Syst Biol*. 2008; 2:4. [PubMed: 18194572]
114. Saez-Rodriguez J, Alexopoulos LG, Epperlein J, Samaga R, Lauffenburger DA, Klamt S, Sorger PK. Discrete logic modelling as a means to link protein signalling networks with functional analysis of mammalian signal transduction. *Mol Syst Biol*. 2009; 5:331. [PubMed: 19953085]
115. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. *Nat Rev Cancer*. 10:116–129. [PubMed: 20094046]
116. Schlessinger J. Common and distinct elements in cellular signaling via EGF and FGF receptors. *Science*. 2004; 306:1506–1507. [PubMed: 15567848]
117. Peters K, Werner S, Liao X, Wert S, Whitsett J, Williams L. Targeted Expression of a Dominant-Negative Fgf Receptor Blocks Branching Morphogenesis and Epithelial Differentiation of the Mouse Lung. *Embo Journal*. 1994; 13:3296–3301. [PubMed: 8045260]
118. Ornitz DM, Itoh N. Fibroblast growth factors. *Genome Biology*. 2001; 2

119. Lowenstein EJ, Daly RJ, Batzer AG, Li W, Margolis B, Lammers R, Ullrich A, Skolnik EY, Bar-Sagi D, Schlessinger J. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell*. 1992; 70:431–442. [PubMed: 1322798]
120. Kouhara H, Hadari YR, Spivak-Kroizman T, Schilling J, Bar-Sagi D, Lax I, Schlessinger J. A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. *Cell*. 1997; 89:693–702. [PubMed: 9182757]
121. Prudovsky I, Savion N, Zhan X, Friesel R, Xu J, Hou J, McKeenan WL, Maciag T. Intact and functional fibroblast growth factor (FGF) receptor-1 trafficks near the nucleus in response to FGF-1. *J Biol Chem*. 1994; 269:31720–31724. [PubMed: 7527394]
122. Zhan X, Plourde C, Hu X, Friesel R, Maciag T. Association of fibroblast growth factor receptor-1 with c-Src correlates with association between c-Src and cortactin. *J Biol Chem*. 1994; 269:20221–20224. [PubMed: 7519605]
123. Sandilands E, Akbarzadeh S, Vecchione A, McEwan DG, Frame MC, Heath JK. Src kinase modulates the activation, transport and signalling dynamics of fibroblast growth factor receptors. *EMBO Rep*. 2007; 8:1162–1169. [PubMed: 17975556]
124. Faeder JR, Blinov ML, Goldstein B, Hlavacek WS. Combinatorial complexity and dynamical restriction of network flows in signal transduction. *Syst Biol (Stevenage)*. 2005; 2:5–15. [PubMed: 17091578]
125. Furdulj CM, Lew ED, Schlessinger J, Anderson KS. Autophosphorylation of FGFR1 kinase is mediated by a sequential and precisely ordered reaction. *Mol Cell*. 2006; 21:711–717. [PubMed: 16507368]
126. Gaffney EA, Heath JK, Kwiatkowska MZ. A mass action model of a Fibroblast Growth Factor signaling pathway and its simplification. *Bull Math Biol*. 2008; 70:2229–2263. [PubMed: 18841420]
127. Cohen S. The stimulation of epidermal proliferation by a specific protein (EGF). *Dev Biol*. 1965; 12:394–407. [PubMed: 5884352]
128. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001; 2:127–137. [PubMed: 11252954]
129. Wiley HS, Shvartsman SY, Lauffenburger DA. Computational modeling of the EGF-receptor system: a paradigm for systems biology. *Trends Cell Biol*. 2003; 13:43–50. [PubMed: 12480339]
130. Schoeberl B, Eichler-Jonsson C, Gilles ED, Muller G. Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors. *Nat Biotechnol*. 2002; 20:370–375. [PubMed: 11923843]
131. Chen WW, Schoeberl B, Jasper PJ, Niepel M, Nielsen UB, Lauffenburger DA, Sorger PK. Input-output behavior of ErbB signaling pathways as revealed by a mass action model trained against dynamic data. *Mol Syst Biol*. 2009; 5:239. [PubMed: 19156131]
132. Nakakuki T, Birtwistle MR, Saeki Y, Yumoto N, Ide K, Nagashima T, Brusch L, Ogunnaike BA, Okada-Hatakeyama M, Kholodenko BN. Ligand-specific c-Fos expression emerges from the spatiotemporal control of ErbB network dynamics. *Cell*. 2010; 141:884–896. [PubMed: 20493519]
133. Koff A, Ohtsuki M, Polyak K, Roberts JM, Massague J. Negative Regulation of G1 in Mammalian-Cells - Inhibition of Cyclin-E-Dependent Kinase by Tgf-Beta. *Science*. 1993; 260:536–539. [PubMed: 8475385]
134. Schmierer B, Hill CS. TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. *Nat Rev Mol Cell Biol*. 2007; 8:970–982. [PubMed: 18000526]
135. Moses HL, Yang EY, Pietenpol JA. TGF-beta stimulation and inhibition of cell proliferation: new mechanistic insights. *Cell*. 1990; 63:245–247. [PubMed: 2208284]
136. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell*. 2003; 113:685–700. [PubMed: 12809600]
137. He W, Li AG, Wang D, Han S, Zheng B, Goumans MJ, Ten Dijke P, Wang XJ. Overexpression of Smad7 results in severe pathological alterations in multiple epithelial tissues. *EMBO J*. 2002; 21:2580–2590. [PubMed: 12032071]

138. Chen F, Weinberg RA. Biochemical evidence for the autophosphorylation and transphosphorylation of transforming growth factor beta receptor kinases. *Proc Natl Acad Sci U S A*. 1995; 92:1565–1569. [PubMed: 7878020]
139. Furuhashi M, Yagi K, Yamamoto H, Furukawa Y, Shimada S, Nakamura Y, Kikuchi A, Miyazono K, Kato M. Axin facilitates Smad3 activation in the transforming growth factor beta signaling pathway. *Mol Cell Biol*. 2001; 21:5132–5141. [PubMed: 11438668]
140. Heldin CH, Miyazono K, tenDijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature*. 1997; 390:465–471. [PubMed: 9393997]
141. Kavsak P, Rasmussen RK, Causing CG, Bonni S, Zhu H, Thomsen GH, Wrana JL. Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF beta receptor for degradation. *Mol Cell*. 2000; 6:1365–1375. [PubMed: 11163210]
142. Koli KM, Arteaga CL. Predominant cytosolic localization of type II transforming growth factor beta receptors in human breast carcinoma cells. *Cancer Res*. 1997; 57:970–977. [PubMed: 9041203]
143. Vilar JMG, Jansen R, Sander C. Signal processing in the TGF-beta superfamily ligand-receptor network. *Plos Computational Biology*. 2006; 2:36–45.
144. Hendriks BS, Opresko LK, Wiley HS, Lauffenburger D. Quantitative analysis of HER2-mediated effects on HER2 and epidermal growth factor receptor endocytosis: distribution of homo- and heterodimers depends on relative HER2 levels. *J Biol Chem*. 2003; 278:23343–23351. [PubMed: 12686539]
145. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*. 2004; 20:781–810. [PubMed: 15473860]
146. Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, Yates JR 3rd, Nusse R. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature*. 2003; 423:448–452. [PubMed: 12717451]
147. Jamora C, DasGupta R, Kocieniewski P, Fuchs E. Links between signal transduction, transcription and adhesion in epithelial bud development. *Nature*. 2003; 422:317–322. [PubMed: 12646922]
148. Stark K, Vainio S, Vassileva G, McMahon AP. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature*. 1994; 372:679–683. [PubMed: 7990960]
149. Briskin C, Heineman A, Chavarria T, Elenbaas B, Tan J, Dey SK, McMahon JA, McMahon AP, Weinberg RA. Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling. *Genes Dev*. 2000; 14:650–654. [PubMed: 10733525]
150. Bhanot P, Brink M, Samos CH, Hsieh JC, Wang Y, Macke JP, Andrew D, Nathans J, Nusse R. A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature*. 1996; 382:225–230. [PubMed: 8717036]
151. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell*. 2006; 127:469–480. [PubMed: 17081971]
152. Tamai K, Semenov M, Kato Y, Spokony R, Liu C, Katsuyama Y, Hess F, Saint-Jeannet JP, He X. LDL-receptor-related proteins in Wnt signal transduction. *Nature*. 2000; 407:530–535. [PubMed: 11029007]
153. Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. *EMBO J*. 1998; 17:1371–1384. [PubMed: 9482734]
154. Behrens J, Jerchow BA, Wurtele M, Grimm J, Asbrand C, Wirtz R, Kuhl M, Wedlich D, Birchmeier W. Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta. *Science*. 1998; 280:596–599. [PubMed: 9554852]
155. Papkoff J, Rubinfeld B, Schryver B, Polakis P. Wnt-1 regulates free pools of catenins and stabilizes APC-catenin complexes. *Mol Cell Biol*. 1996; 16:2128–2134. [PubMed: 8628279]
156. Xing Y, Clements WK, Le Trong I, Hinds TR, Stenkamp R, Kimelman D, Xu W. Crystal structure of a beta-catenin/APC complex reveals a critical role for APC phosphorylation in APC function. *Mol Cell*. 2004; 15:523–533. [PubMed: 15327769]

157. Peters JM, McKay RM, McKay JP, Graff JM. Casein kinase I transduces Wnt signals. *Nature*. 1999; 401:345–350. [PubMed: 10517632]
158. Zeng X, Tamai K, Doble B, Li S, Huang H, Habas R, Okamura H, Woodgett J, He X. A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature*. 2005; 438:873–877. [PubMed: 16341017]
159. Kimelman D, Xu W. beta-catenin destruction complex: insights and questions from a structural perspective. *Oncogene*. 2006; 25:7482–7491. [PubMed: 17143292]
160. Yang-Snyder J, Miller JR, Brown JD, Lai CJ, Moon RT. A frizzled homolog functions in a vertebrate Wnt signaling pathway. *Curr Biol*. 1996; 6:1302–1306. [PubMed: 8939578]
161. Zeng X, Huang H, Tamai K, Zhang X, Harada Y, Yokota C, Almeida K, Wang J, Doble B, Woodgett J, et al. Initiation of Wnt signaling: control of Wnt coreceptor Lrp6 phosphorylation/activation via frizzled, dishevelled and axin functions. *Development*. 2008; 135:367–375. [PubMed: 18077588]
162. Giese K, Kingsley C, Kirshner JR, Grosschedl R. Assembly and function of a TCR alpha enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein-protein interactions. *Genes Dev*. 1995; 9:995–1008. [PubMed: 7774816]
163. Huber O, Korn R, McLaughlin J, Ohsugi M, Herrmann BG, Kemler R. Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. *Mech Dev*. 1996; 59:3–10. [PubMed: 8892228]
164. Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W. Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature*. 1996; 382:638–642. [PubMed: 8757136]
165. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science*. 1997; 275:1787–1790. [PubMed: 9065402]
166. Salic A, Lee E, Mayer L, Kirschner MW. Control of beta-catenin stability: reconstitution of the cytoplasmic steps of the wnt pathway in *Xenopus* egg extracts. *Mol Cell*. 2000; 5:523–532. [PubMed: 10882137]
167. Lee E, Salic A, Kruger R, Heinrich R, Kirschner MW. The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. *PLoS Biol*. 2003; 1:E10. [PubMed: 14551908]
168. Tolwinski NS, Wehrli M, Rives A, Erdeniz N, DiNardo S, Wieschaus E. Wg/Wnt signal can be transmitted through arrow/LRP5,6 and Axin independently of Zw3/Gsk3beta activity. *Dev Cell*. 2003; 4:407–418. [PubMed: 12636921]
169. Yamamoto H, Kishida S, Kishida M, Ikeda S, Takada S, Kikuchi A. Phosphorylation of axin, a Wnt signal negative regulator, by glycogen synthase kinase-3beta regulates its stability. *J Biol Chem*. 1999; 274:10681–10684. [PubMed: 10196136]
170. Kirschner MW. The meaning of systems biology. *Cell*. 2005; 121:503–504. [PubMed: 15907462]
171. Gumbiner BM. Regulation of cadherin-mediated adhesion in morphogenesis. *Nat Rev Mol Cell Biol*. 2005; 6:622–634. [PubMed: 16025097]
172. Yamada S, Nelson WJ. Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. *J Cell Biol*. 2007; 178:517–527. [PubMed: 17646397]
173. Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer Res*. 2008; 68:3645–3654. [PubMed: 18483246]
174. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell*. 2008; 14:818–829. [PubMed: 18539112]
175. Peinado H, Marin F, Cubillo E, Stark HJ, Fusenig N, Nieto MA, Cano A. Snail and E47 repressors of E-cadherin induce distinct invasive and angiogenic properties in vivo. *J Cell Sci*. 2004; 117:2827–2839. [PubMed: 15169839]
176. Wang X, Zheng M, Liu G, Xia W, McKeown-Longo PJ, Hung MC, Zhao J. Kruppel-like factor 8 induces epithelial to mesenchymal transition and epithelial cell invasion. *Cancer Res*. 2007; 67:7184–7193. [PubMed: 17671186]

177. Sobrado VR, Moreno-Bueno G, Cubillo E, Holt LJ, Nieto MA, Portillo F, Cano A. The class I bHLH factors E2-2A and E2-2B regulate EMT. *J Cell Sci.* 2009; 122:1014–1024. [PubMed: 19295128]
178. Ramis-Conde I, Drasdo D, Anderson AR, Chaplain MA. Modeling the influence of the E-cadherin-beta-catenin pathway in cancer cell invasion: a multiscale approach. *Biophys J.* 2008; 95:155–165. [PubMed: 18339758]
179. Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Lochner D, Birchmeier W. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol.* 1991; 113:173–185. [PubMed: 2007622]
180. Bessho Y, Kageyama R. Oscillations, clocks and segmentation. *Curr Opin Genet Dev.* 2003; 13:379–384. [PubMed: 12888011]
181. Dale JK, Maroto M, Dequeant ML, Malapert P, McGrew M, Pourquie O. Periodic notch inhibition by lunatic fringe underlies the chick segmentation clock. *Nature.* 2003; 421:275–278. [PubMed: 12529645]
182. Mazzone M, Selfors LM, Albeck J, Overholtzer M, Sale S, Carroll DL, Pandya D, Lu Y, Mills GB, Aster JC, et al. Dose-dependent induction of distinct phenotypic responses to Notch pathway activation in mammary epithelial cells. *Proc Natl Acad Sci U S A.* 2010; 107:5012–5017. [PubMed: 20194747]
183. Stylianou S, Clarke RB, Brennan K. Aberrant activation of notch signaling in human breast cancer. *Cancer Res.* 2006; 66:1517–1525. [PubMed: 16452208]
184. Webb SD, Owen MR. Oscillations and patterns in spatially discrete models for developmental intercellular signalling. *J Math Biol.* 2004; 48:444–476. [PubMed: 15052506]
185. Kimble J, Simpson P. The LIN-12/Notch signaling pathway and its regulation. *Annu Rev Cell Dev Biol.* 1997; 13:333–361. [PubMed: 9442877]
186. de Celis JF, Bray S. Feed-back mechanisms affecting Notch activation at the dorsoventral boundary in the *Drosophila* wing. *Development.* 1997; 124:3241–3251. [PubMed: 9310319]
187. Jorgensen C, Sherman A, Chen GI, Pasculescu A, Poliakov A, Hsiung M, Larsen B, Wilkinson DG, Lindring R, Pawson T. Cell-specific information processing in segregating populations of Eph receptor ephrin-expressing cells. *Science.* 2009; 326:1502–1509. [PubMed: 20007894]
188. Battle E, Bacani J, Begthel H, Jonkheer S, Gregorieff A, van de Born M, Malats N, Sancho E, Boon E, Pawson T, et al. EphB receptor activity suppresses colorectal cancer progression. *Nature.* 2005; 435:1126–1130. [PubMed: 15973414]
189. Cortina C, Palomo-Ponce S, Iglesias M, Fernandez-Masip JL, Vivancos A, Whissell G, Huma M, Peiro N, Gallego L, Jonkheer S, et al. EphB-ephrin-B interactions suppress colorectal cancer progression by compartmentalizing tumor cells. *Nat Genet.* 2007; 39:1376–1383. [PubMed: 17906625]
190. Gehler S, Baldassarre M, Lad Y, Leight JL, Wozniak MA, Riching KM, Eliceiri KW, Weaver VM, Calderwood DA, Keely PJ. Filamin A-beta1 integrin complex tunes epithelial cell response to matrix tension. *Mol Biol Cell.* 2009; 20:3224–3238. [PubMed: 19458194]
191. Alexander NR, Branch KM, Parekh A, Clark ES, Iwueke IC, Guelcher SA, Weaver AM. Extracellular matrix rigidity promotes invadopodia activity. *Curr Biol.* 2008; 18:1295–1299. [PubMed: 18718759]
192. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, et al. Tensional homeostasis and the malignant phenotype. *Cancer Cell.* 2005; 8:241–254. [PubMed: 16169468]
193. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell.* 2009; 139:891–906. [PubMed: 19931152]
194. Gudjonsson T, Villadsen R, Nielsen HL, Ronnov-Jessen L, Bissell MJ, Petersen OW. Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties. *Genes Dev.* 2002; 16:693–706. [PubMed: 11914275]
195. Villadsen R, Fridriksdottir AJ, Ronnov-Jessen L, Gudjonsson T, Rank F, LaBarge MA, Bissell MJ, Petersen OW. Evidence for a stem cell hierarchy in the adult human breast. *J Cell Biol.* 2007; 177:87–101. [PubMed: 17420292]

196. Asselin-Labat ML, Sutherland KD, Barker H, Thomas R, Shackleton M, Forrest NC, Hartley L, Robb L, Grosveld FG, van der Wees J, et al. Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. *Nat Cell Biol.* 2007; 9:201–209. [PubMed: 17187062]
197. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, Asselin-Labat ML, Gyorki DE, Ward T, Partanen A, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nat Med.* 2009; 15:907–913. [PubMed: 19648928]
198. Kouros-Mehr H, Slorach EM, Sternlicht MD, Werb Z. GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland. *Cell.* 2006; 127:1041–1055. [PubMed: 17129787]
199. Visvader JE. Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Genes Dev.* 2009; 23:2563–2577. [PubMed: 19933147]
200. van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol.* 2009; 71:241–260. [PubMed: 18808327]
201. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, et al. Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature.* 2007; 449:1003–1007. [PubMed: 17934449]
202. Zhu L, Gibson P, Currie DS, Tong Y, Richardson RJ, Bayazitov IT, Poppleton H, Zakharenko S, Ellison DW, Gilbertson RJ. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature.* 2009; 457:603–607. [PubMed: 19092805]
203. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, et al. Single *Lgr5* stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature.* 2009; 459:262–265. [PubMed: 19329995]
204. Sangiorgi E, Capecchi MR. *Bmi1* is expressed in vivo in intestinal stem cells. *Nat Genet.* 2008; 40:915–920. [PubMed: 18536716]
205. Snippert HJ, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, Barker N, Klein AM, van Rheenen J, Simons BD, et al. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing *Lgr5* stem cells. *Cell.* 2010; 143:134–144. [PubMed: 20887898]
206. Lopez-Garcia C, Klein AM, Simons BD, Winton DJ. Intestinal stem cell replacement follows a pattern of neutral drift. *Science.* 2010; 330:822–825. [PubMed: 20929733]
207. Janes KA, Wang CC, Holmberg KJ, Cabral K, Brugge JS. Identifying single-cell molecular programs by stochastic profiling. *Nat Methods.* 2010; 7:311–317. [PubMed: 20228812]
208. Weinstein IB. Cancer. Addiction to oncogenes--the Achilles heel of cancer. *Science.* 2002; 297:63–64. [PubMed: 12098689]
209. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell.* 2009; 136:823–837. [PubMed: 19269363]
210. Wang F, Hansen RK, Radisky D, Yoneda T, Barcellos-Hoff MH, Petersen OW, Turley EA, Bissell MJ. Phenotypic reversion or death of cancer cells by altering signaling pathways in three-dimensional contexts. *J Natl Cancer Inst.* 2002; 94:1494–1503. [PubMed: 12359858]
211. Barcellos-Hoff MH, Ravani SA. Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. *Cancer Res.* 2000; 60:1254–1260. [PubMed: 10728684]
212. Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, Damsky C, Bissell MJ. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J Cell Biol.* 1997; 137:231–245. [PubMed: 9105051]
213. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* 2000; 100:57–70. [PubMed: 10647931]
214. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011; 144:646–674. [PubMed: 21376230]
215. Hay ED. An overview of epithelio-mesenchymal transformation. *Acta Anat (Basel).* 1995; 154:8–20. [PubMed: 8714286]
216. Oft M, Akhurst RJ, Balmain A. Metastasis is driven by sequential elevation of H-ras and Smad2 levels. *Nat Cell Biol.* 2002; 4:487–494. [PubMed: 12105419]

217. Hogan C, Dupre-Crochet S, Norman M, Kajita M, Zimmermann C, Pelling AE, Piddini E, Baena-Lopez LA, Vincent JP, Itoh Y, et al. Characterization of the interface between normal and transformed epithelial cells. *Nat Cell Biol.* 2009; 11:460–467. [PubMed: 19287376]
218. Muthuswamy SK, Li D, Lelievre S, Bissell MJ, Brugge JS. ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. *Nat Cell Biol.* 2001; 3:785–792. [PubMed: 11533657]
219. Schafer ZT, Grassian AR, Song L, Jiang Z, Gerhart-Hines Z, Irie HY, Gao S, Puigserver P, Brugge JS. Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment. *Nature.* 2009; 461:109–113. [PubMed: 19693011]
220. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 2009; 324:1029–1033. [PubMed: 19460998]
221. Owens LV, Xu L, Craven RJ, Dent GA, Weiner TM, Kornberg L, Liu ET, Cance WG. Overexpression of the focal adhesion kinase (p125FAK) in invasive human tumors. *Cancer Res.* 1995; 55:2752–2755. [PubMed: 7796399]
222. Benlimame N, He Q, Jie S, Xiao D, Xu YJ, Loignon M, Schlaepfer DD, Alaoui-Jamali MA. FAK signaling is critical for ErbB-2/ErbB-3 receptor cooperation for oncogenic transformation and invasion. *J Cell Biol.* 2005; 171:505–516. [PubMed: 16275754]
223. Hsia DA, Mitra SK, Hauck CR, Streblow DN, Nelson JA, Ilic D, Huang S, Li E, Nemerow GR, Leng J, et al. Differential regulation of cell motility and invasion by FAK. *J Cell Biol.* 2003; 160:753–767. [PubMed: 12615911]
224. Wu X, Gan B, Yoo Y, Guan JL. FAK-mediated src phosphorylation of endophilin A2 inhibits endocytosis of MT1-MMP and promotes ECM degradation. *Dev Cell.* 2005; 9:185–196. [PubMed: 16054026]
225. Avizienyte E, Frame MC. Src and FAK signalling controls adhesion fate and the epithelial-to-mesenchymal transition. *Curr Opin Cell Biol.* 2005; 17:542–547. [PubMed: 16099634]
226. Cicchini C, Laudadio I, Citarella F, Corazzari M, Steindler C, Conigliaro A, Fantoni A, Amicone L, Tripodi M. TGFbeta-induced EMT requires focal adhesion kinase (FAK) signaling. *Exp Cell Res.* 2008; 314:143–152. [PubMed: 17949712]
227. van Nimwegen MJ, van de Water B. Focal adhesion kinase: a potential target in cancer therapy. *Biochem Pharmacol.* 2007; 73:597–609. [PubMed: 16997283]
228. Lim ST, Chen XL, Tomar A, Miller NL, Yoo J, Schlaepfer DD. Knock-in mutation reveals an essential role for focal adhesion kinase activity in blood vessel morphogenesis and cell motility-polarity but not cell proliferation. *J Biol Chem.* 2010; 285:21526–21536. [PubMed: 20442405]
229. Brent R. A partnership between biology and engineering. *Nat Biotechnol.* 2004; 22:1211–1214. [PubMed: 15470452]
230. Yamada KM, Cukierman E. Modeling tissue morphogenesis and cancer in 3D. *Cell.* 2007; 130:601–610. [PubMed: 17719539]
231. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. *Semin Cancer Biol.* 2005; 15:378–386. [PubMed: 15975825]
232. Pampaloni F, Reynaud EG, Stelzer EH. The third dimension bridges the gap between cell culture and live tissue. *Nat Rev Mol Cell Biol.* 2007; 8:839–845. [PubMed: 17684528]
233. Montesano R, Schaller G, Orci L. Induction of epithelial tubular morphogenesis in vitro by fibroblast-derived soluble factors. *Cell.* 1991; 66:697–711. [PubMed: 1878968]
234. Wang AZ, Ojakian GK, Nelson WJ. Steps in the morphogenesis of a polarized epithelium. II. Disassembly and assembly of plasma membrane domains during reversal of epithelial cell polarity in multicellular epithelial (MDCK) cysts. *J Cell Sci.* 1990; 95(Pt 1):153–165. [PubMed: 2351700]
235. Bryant DM, Datta A, Rodriguez-Fraticelli AE, Peranen J, Martin-Belmonte F, Mostov KE. A molecular network for de novo generation of the apical surface and lumen. *Nat Cell Biol.* 2010; 12:1035–1045. [PubMed: 20890297]
236. Saxen L, Sariola H. Early organogenesis of the kidney. *Pediatr Nephrol.* 1987; 1:385–392. [PubMed: 3153305]

237. McAteer JA, Evan AP, Gardner KD. Morphogenetic clonal growth of kidney epithelial cell line MDCK. *Anat Rec.* 1987; 217:229–239. [PubMed: 3578840]
238. Chen X, Macara IG. RNA interference techniques to study epithelial cell adhesion and polarity. *Methods Enzymol.* 2006; 406:362–374. [PubMed: 16472670]
239. Debnath J, Muthuswamy SK, Brugge JS. Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods.* 2003; 30:256–268. [PubMed: 12798140]
240. Hariharan IK, Haber DA. Yeast, flies, worms, and fish in the study of human disease. *N Engl J Med.* 2003; 348:2457–2463. [PubMed: 12802034]
241. Moffat J, Grueneberg DA, Yang X, Kim SY, Kloepper AM, Hinkle G, Piqani B, Eisenhaure TM, Luo B, Grenier JK, et al. A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. *Cell.* 2006; 124:1283–1298. [PubMed: 16564017]
242. Schluter MA, Pfarr CS, Pieczynski J, Whiteman EL, Hurd TW, Fan SL, Liu CJ, Margolis B. Trafficking of Crumbs3 during Cytokinesis Is Crucial for Lumen Formation. *Molecular Biology of the Cell.* 2009; 20:4652–4663. [PubMed: 19776356]
243. Aranda V, Haire T, Nolan ME, Calarco JP, Rosenberg AZ, Fawcett JP, Pawson T, Muthuswamy SK. Par6-aPKC uncouples ErbB2 induced disruption of polarized epithelial organization from proliferation control. *Nat Cell Biol.* 2006; 8:1235–1245. [PubMed: 17060907]
244. Richard NR, Anderson JA, Weiss JL, Binder PS. Air/liquid corneal organ culture: a light microscopic study. *Curr Eye Res.* 1991; 10:739–749. [PubMed: 1914506]
245. Foreman DM, Pancholi S, Jarvis-Evans J, McLeod D, Boulton ME. A simple organ culture model for assessing the effects of growth factors on corneal re-epithelialization. *Exp Eye Res.* 1996; 62:555–564. [PubMed: 8759523]
246. Xu KP, Li XF, Yu FS. Corneal organ culture model for assessing epithelial responses to surfactants. *Toxicol Sci.* 2000; 58:306–314. [PubMed: 11099643]
247. Lu Z, Hasse S, Bodo E, Rose C, Funk W, Paus R. Towards the development of a simplified long-term organ culture method for human scalp skin and its appendages under serum-free conditions. *Exp Dermatol.* 2007; 16:37–44. [PubMed: 17181635]
248. Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M, Clevers H. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature.* 2010
249. Nelson CM, Inman JL, Bissell MJ. Three-dimensional lithographically defined organotypic tissue arrays for quantitative analysis of morphogenesis and neoplastic progression. *Nat Protoc.* 2008; 3:674–678. [PubMed: 18388950]
250. Elowitz M, Lim WA. Build life to understand it. *Nature.* 2010; 468:889–890. [PubMed: 21164460]
251. Rejniak KA, Anderson AR. Hybrid models of tumor growth. *Wiley Interdiscip Rev Syst Biol Med.* 2011; 3:115–125. [PubMed: 21064037]
252. Edelman LB, Eddy JA, Price ND. In silico models of cancer. *Wiley Interdiscip Rev Syst Biol Med.* 2010; 2:438–459. [PubMed: 20836040]
253. Smallwood R. Computational modeling of epithelial tissues. *Wiley Interdiscip Rev Syst Biol Med.* 2009; 1:191–201. [PubMed: 20835991]
254. Turing AM. The chemical basis of morphogenesis. *Proc R Soc Lond B Biol Sci.* 1952; 237:37–72.
255. Franks SJ, Byrne HM, Underwood JC, Lewis CE. Biological inferences from a mathematical model of comedo ductal carcinoma in situ of the breast. *J Theor Biol.* 2005; 232:523–543. [PubMed: 15588633]
256. Sherratt JA, Dallon JC. Theoretical models of wound healing: past successes and future challenges. *C R Biol.* 2002; 325:557–564. [PubMed: 12187641]
257. Mi Q, Swigon D, Riviere B, Cetin S, Vodovotz Y, Hackam DJ. One-dimensional elastic continuum model of enterocyte layer migration. *Biophys J.* 2007; 93:3745–3752. [PubMed: 17704181]
258. Edwards CM, Chapman SJ. Biomechanical modelling of colorectal crypt budding and fission. *Bull Math Biol.* 2007; 69:1927–1942. [PubMed: 17443390]

259. Fisher RA. The wave of advance of advantageous genes. *Ann Eugenics*. 1937; 7:353–369.
260. Savla U, Olson LE, Waters CM. Mathematical modeling of airway epithelial wound closure during cyclic mechanical strain. *J Appl Physiol*. 2004; 96:566–574. [PubMed: 14715680]
261. Haugh JM. Deterministic model of dermal wound invasion incorporating receptor-mediated signal transduction and spatial gradient sensing. *Biophys J*. 2006; 90:2297–2308. [PubMed: 16415056]
262. Wolpert L. Positional information and the spatial pattern of cellular differentiation. *J Theor Biol*. 1969; 25:1–47. [PubMed: 4390734]
263. Kim SH, Yu W, Mostov K, Matthay MA, Hunt CA. A computational approach to understand in vitro alveolar morphogenesis. *PLoS One*. 2009; 4:e4819. [PubMed: 19283073]
264. Kim SH, Matthay MA, Mostov K, Hunt CA. Simulation of lung alveolar epithelial wound healing in vitro. *J R Soc Interface*. 2010; 7:1157–1170. [PubMed: 20236957]
265. Grant MR, Mostov KE, Tlsty TD, Hunt CA. Simulating properties of in vitro epithelial cell morphogenesis. *PLoS Comput Biol*. 2006; 2:e129. [PubMed: 17029556]
266. Walker DC, Hill G, Wood SM, Smallwood RH, Southgate J. Agent-based computational modeling of wounded epithelial cell monolayers. *IEEE Trans Nanobioscience*. 2004; 3:153–163. [PubMed: 15473067]
267. Walker DC, Georgopoulos NT, Southgate J. Anti-social cells: predicting the influence of E-cadherin loss on the growth of epithelial cell populations. *J Theor Biol*. 2010; 262:425–440. [PubMed: 19852973]
268. Rejniak KA. A single-cell approach in modeling the dynamics of tumor microregions. *Math Biosci Eng*. 2005; 2:643–655. [PubMed: 20369945]
269. Rejniak KA, Anderson AR. A computational study of the development of epithelial acini: I. Sufficient conditions for the formation of a hollow structure. *Bull Math Biol*. 2008; 70:677–712. [PubMed: 18188652]
270. Rejniak KA, Anderson AR. A computational study of the development of epithelial acini: II. Necessary conditions for structure and lumen stability. *Bull Math Biol*. 2008; 70:1450–1479. [PubMed: 18401665]
271. Rejniak KA, Wang SE, Bryce NS, Chang H, Parvin B, Jourquin J, Estrada L, Gray JW, Arteaga CL, Weaver AM, et al. Linking changes in epithelial morphogenesis to cancer mutations using computational modeling. *PLoS Comput Biol*. 2010;6.
272. Wang SE, Narasanna A, Perez-Torres M, Xiang B, Wu FY, Yang S, Carpenter G, Gazdar AF, Muthuswamy SK, Arteaga CL. HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. *Cancer Cell*. 2006; 10:25–38. [PubMed: 16843263]
273. Gevertz J, Torquato S. Growing heterogeneous tumors in silico. *Phys Rev E Stat Nonlin Soft Matter Phys*. 2009; 80:051910. [PubMed: 20365009]
274. Cickovski T, Aras K, Alber MS, Izaguirre JA, Swat M, Glazier JA, Merks RM, Glimm T, Hentschel HG, Newman SA. From Genes to Organisms Via the Cell A Problem-Solving Environment for Multicellular Development. *Comput Sci Eng*. 2007; 9:50–60. [PubMed: 19526065]
275. Walker D, Wood S, Southgate J, Holcombe M, Smallwood R. An integrated agent-mathematical model of the effect of intercellular signalling via the epidermal growth factor receptor on cell proliferation. *J Theor Biol*. 2006; 242:774–789. [PubMed: 16765384]
276. Anderson AR, C M, Newman EL, Steele RJ, Thompson AM. Mathematical Modelling of Tumour Invasion and Metastasis. *J Theoret Med*. 2000; 2:129–154.
277. Anderson AR. A hybrid mathematical model of solid tumour invasion: the importance of cell adhesion. *Math Med Biol*. 2005; 22:163–186. [PubMed: 15781426]
278. Anderson AR, Weaver AM, Cummings PT, Quaranta V. Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment. *Cell*. 2006; 127:905–915. [PubMed: 17129778]
279. Gerlee P, Anderson AR. An evolutionary hybrid cellular automaton model of solid tumour growth. *J Theor Biol*. 2007; 246:583–603. [PubMed: 17374383]

280. Rejniak KA. An immersed boundary framework for modelling the growth of individual cells: an application to the early tumour development. *J Theor Biol.* 2007; 247:186–204. [PubMed: 17416390]
281. Anderson AR, Hassanein M, Branch KM, Lu J, Lobdell NA, Maier J, Basanta D, Weidow B, Narasanna A, Arteaga CL, et al. Microenvironmental independence associated with tumor progression. *Cancer Res.* 2009; 69:8797–8806. [PubMed: 19887618]
282. Anderson AR, Rejniak KA, Gerlee P, Quaranta V. Microenvironment driven invasion: a multiscale multimodel investigation. *J Math Biol.* 2009; 58:579–624. [PubMed: 18839176]
283. Jeon J, Quaranta V, Cummings PT. An off-lattice hybrid discrete-continuum model of tumor growth and invasion. *Biophys J.* 2010; 98:37–47. [PubMed: 20074513]
284. Schoeberl B, Pace E, Howard S, Garantcharova V, Kudla A, Sorger PK, Nielsen UB. A data-driven computational model of the ErbB receptor signaling network. *Conf Proc IEEE Eng Med Biol Soc.* 2006; 1:53–54. [PubMed: 17946779]
285. Janes KA, Reinhardt HC, Yaffe MB. Cytokine-induced signaling networks prioritize dynamic range over signal strength. *Cell.* 2008; 135:343–354. [PubMed: 18957207]
286. Miller-Jensen K, Janes KA, Brugge JS, Lauffenburger DA. Common effector processing mediates cell-specific responses to stimuli. *Nature.* 2007; 448:604–608. [PubMed: 17637676]

FIGURE 1.

Morphology of a polarized epithelial monolayer. (a) Vertical epithelial polarization into distinct apical, basal, and lateral plasma membrane domains. Apical surfaces contain specialized microvilli for absorption and secretion. Basal surfaces bind to extracellular matrix (ECM) proteins via integrins to assemble focal adhesions. Lateral membranes contain junctional complexes (tight junctions, adherens junctions, and desmosomes) that link the adjacent cells and provide diffusion barriers. (b) Horizontal epithelial polarization of core planar cell polarity (PCP) proteins. In the *Drosophila* wing, *fz*, *dsh*, and *dgo* localize to the distal cortical domains, whereas *pk* and *Vang* localize to the proximal cortical domains. Shortly after the maximal asymmetry of PCP proteins is observed, actin-rich prehairsts initiate from the distal domain of the cells. (c) Intracellular tension and intercellular forces shape epithelial morphology. Apical constriction results from the counterbalance of cortical tension and cellular adhesion. This constriction contributes line tensions at the interface between cells and serves as an energy barrier to cellular rearrangements.

FIGURE 2.

Building epithelial tissue architecture from tubes, branches, and acini. (a) Epithelial tubes are cylindrical monolayers with inner apical surfaces and outside basal surfaces that surround a central lumen. (b) Branched tubular networks result from epithelial extensions and bifurcations into the surrounding stroma. (c) Hollow spherical acini often terminate complex networks of branched tubes.

FIGURE 3.

Diffusible factors involved in epithelial morphogenesis. (a) Hepatocyte growth factor (HGF) signaling. Ligand binding to the HGF receptor (HGFR) leads to tyrosine autophosphorylation and Ras–MAPK (mitogen-activated protein kinase) signaling. (b) Fibroblast growth factor (FGF) signaling. Ligand binding to the FGF receptor (FGFR) leads to tyrosine autophosphorylation and recruitment of fibroblast growth factor receptor substrate 2 (FRS2) to activate Ras–MAPK and phosphoinositide 3-kinase (PI3K)–AKT signaling. (c) Epidermal growth factor (EGF) signaling. Ligand binding to ErbB receptors leads to homo- and heterodimerization, tyrosine autophosphorylation, and signaling through the MAPK and AKT pathways. ErbB2 does not bind EGF-family ligands, and ErbB3 is catalytically inactive. (d) Transforming growth factor β (TGF β) signaling. Ligand binding leads to heterodimerization of TGF β receptor I (TGF β RI) and TGF β receptor II (TGF β RII). TGF β RII transphosphorylates TGF β RI, which phosphorylates SMAD2/3 allowing them to heterodimerize with SMAD4 and translocate into the nucleus to modulate gene expression. (e) Wnt signaling. Ligand binding to the frizzled (Fz) receptor recruits the low-density lipoprotein receptor-related protein (LRP) coreceptor, which downregulates the β -catenin destruction complex. This results in β -catenin accumulation in the cytoplasm and its translocation to nucleus where it binds to T-cell factor/lymphoid enhancer factor (TCF/LEF) and initiates transcription.

FIGURE 4.

Intercellular communication between adjacent epithelial cells via cell–cell signaling receptors. (a) E-cadherin signaling. E-cadherins form extracellular homophilic complexes between neighboring cells. p120 catenin binds to the juxtamembrane domain of E-cadherin and stabilizes E-cadherin by preventing clathrin-mediated endocytosis. β -catenin binds to E-cadherin. α -catenin shuttles between β -catenin and epithelial protein lost in neoplasm (EPLIN), and connects the E-cadherin complexes to F-actin. (b) Delta–Notch signaling. Notch binds to its ligand (Delta or Jagged) on adjacent cells, proteolytically releasing the Notch intracellular domain (NICD). NICD moves to the nucleus and interacts with DNA-binding protein RBP (recombination signal-binding protein for immunoglobulin kappa J region) along with several coactivators to regulate gene expression. Multiple Notch target genes, such as Hairy/enhancer of split (Hes) and lunatic fringe (Lfng), are negative regulators of the Notch pathway. These feedbacks can give rise to oscillations in gene expression. (c) Eph–ephrin signaling. Eph–ephrin binding on neighboring cells leads to bidirectional phosphotyrosine signaling. Eph-expressing cells activate Eph RTK activity together with other tyrosine kinases, such as ABL, for each forward signaling. In ephrin-expressing cells, other RTKs such as insulin-like growth factor receptor (IGF1R) are transactivated. Ephrin-induced reverse signaling is critical for boundary formation.

FIGURE 5.

A subset of the molecular and phenotypic heterogeneities among epithelia in the breast and small intestine. (a) Breast tissue contains an outer layer of myoepithelial cells and an inner layer of luminal epithelia. Stem/progenitor cells are distributed throughout the organ and multiple stem cells can contribute to branching morphogenesis. (b) The crypt–villus unit of the small intestine. Paneth cells lie at the bottom of the crypt. Stem cells located above paneth cells produce transit-amplifying precursors that migrate and differentiate to enterocytes, enteroendocrine cells, goblet cells, or paneth cells.

Table 1

List of abbreviations used

ADAMTS	a disintegrin and metalloproteinase with thrombospondin repeats
APC	adenomatous polyposis coli tumor suppressor
AT II	alveolar type II
c-FLIP	cellular caspase-8 (FLICE)-like inhibitory protein
CK1	casein kinase 1
ECM	extracellular matrix
EGF	epidermal growth factor
EMT	epithelial-to-mesenchymal transition
FAK	focal adhesion kinase
FGF	fibroblast growth factor
FRS2	fibroblast growth factor receptor substrate 2
Gab1	Grb2-associated binding protein 1
GAP	GTPase activating protein
GDNF	glial cell line-derived neurotrophic factor
GEF	guanine nucleotide exchange factor
Grb2	growth factor receptor binding protein 2
GSK3 β	glycogen synthase kinase β
Hes	Hairy/enhancer of split
HGF	hepatocyte growth factor
HSPG	heparin sulfate proteoglycan
LRP	low-density lipoprotein receptor-related protein
MAPK	mitogen-activated protein kinase
MDCK	Madin-Darby canine kidney
MMP	matrix metalloproteinase
MT1-MMP	membrane type 1 matrix metalloproteinase
PCP	planar cell polarity
PI3K	phosphoinositide 3-kinase
ROCK	Rho kinase
RTK	receptor tyrosine kinase
SOS	son-of-sevenless homolog protein
TEB	terminal end bud
TGF β	transforming growth factor β
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand

Table 2Links between core processes for epithelial morphogenesis and the hallmarks of cancer^{213, 214}

Cancer hallmark	Morphogenetic defect	Misregulated core process
Sustaining proliferative signaling ²¹³	<ul style="list-style-type: none"> Excess proliferation Ductal hyperplasia Constitutive growth factor signaling Increased tissue tension 	<ul style="list-style-type: none"> Epithelial cell output Epithelial morphologies – acini Epithelial cell input – diffusible factors Epithelial cell input – substrate stiffness
Resisting cell death ²¹³	<ul style="list-style-type: none"> Insufficient apoptosis Luminal filling Constitutive growth factor signaling 	<ul style="list-style-type: none"> Epithelial cell output Epithelial morphologies – acini Epithelial cell input – diffusible factors
Evading growth suppressors ²¹³	<ul style="list-style-type: none"> Altered responses to TGFβ 	<ul style="list-style-type: none"> Epithelial cell input – diffusible factors
Activating invasion and metastasis ²¹³	<ul style="list-style-type: none"> Disruption of basement membrane Tumor dissemination Constitutive TGFβ signaling E-cadherin loss 	<ul style="list-style-type: none"> ECM remodeling Single cell migration Epithelial cell input – diffusible factors Epithelial cell input – paracellular factors
Genome instability and mutation ²¹⁴	<ul style="list-style-type: none"> Excess cell-to-cell variation 	<ul style="list-style-type: none"> Epithelial heterogeneity

Table 3

Strengths and limitations of different modeling approaches

Approach	Strengths	Limitations
Continuous modeling	<ul style="list-style-type: none"> • Good approximation for many tissue-scale systems • Easier to incorporate experimental data as model parameters. • Fast numerical solvers can be applied to the model • Model properties can be explored by mathematical or numerical analysis 	<ul style="list-style-type: none"> • Unable to track the behavior of individual cells • Unable to capture discrete stochastic events
Discrete modeling	<ul style="list-style-type: none"> • Describes the behavior of single cells in heterogeneous cell populations. • Complex biological processes (such as signaling pathways) can be cast as simple rules in the model. 	<ul style="list-style-type: none"> • Computational cost increases rapidly with the number of cells modeled simultaneously • Cumbersome to handle continuous variables • Many simulations are required to capture stochastic outcomes
Hybrid modeling	<ul style="list-style-type: none"> • Molecular, cellular, and tissue elements can be described in the same model. 	<ul style="list-style-type: none"> • Computational cost limits the number of cells modeled