Looking beyond the genes: the interplay between signaling pathways and mechanics in the shaping and diversification of epithelial tissues

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DOI: https://doi.org/10.1016/bs.ctdb.2016.03.005

Originally published at:
Urdy, Severine; Goudemand, Nicolas; Pantalacci, Sophie (2016). Looking beyond the genes: the interplay between signaling pathways and mechanics in the shaping and diversification of epithelial tissues. In: Orgogozo, Virginie. Genes and Evolution. Cambridge: Elsevier, 227-290.
DOI: https://doi.org/10.1016/bs.ctdb.2016.03.005

Looking Beyond the Genes: The Interplay Between Signaling Pathways and Mechanics in the Shaping and Diversification of Epithelial Tissues

How to cite this paper:
S. Urdy, N. Goudemand, S. Pantalacci, Chapter Seven - Looking Beyond the Genes: The Interplay Between Signaling Pathways and Mechanics in the Shaping and Diversification of Epithelial Tissues, In: Virginie Orgogozo, Editor(s), Current Topics in Developmental Biology, Academic Press, 2016, Volume 119, Pages 227-290, ISSN 0070-2153, ISBN 9780124171947, http://dx.doi.org/10.1016/bs.ctdb.2016.03.005. (http://www.sciencedirect.com/science/article/pii/S0070215316300977)

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CONTENTS

Abstract
The core of Evo-Devo lies in the intuition that the way tissues grow during embryonic development, the way they sustain their structure and function throughout lifetime, and the way they evolve are closely linked. Epithelial tissues are ubiquitous in metazoans, covering the gut and internal branched organs, as well as the skin and its derivatives (ie, teeth). Here, we discuss in vitro, in vivo, and in silico studies on epithelial tissues to illustrate the conserved, dynamical, and complex aspects of their development. We then explore the implications of the dynamical and nonlinear nature of development on the evolution of their size and shape at the phenotypic and genetic levels. In rare cases, when the interplay between signaling and mechanics is well understood at the cell level, it is becoming clear that the structure of development leads to covariation of characters, an integration which in turn provides some predictable structure to evolutionary changes. We suggest that such nonlinear systems are prone to genetic drift, cryptic genetic variation, and context-dependent mutational effects. We argue that experimental and theoretical studies at the cell level are critical to our understanding of the phenotypic and genetic evolution of epithelial tissues, including carcinomas.

Keywords: Morphogenesis; Evo-Devo; Morphodynamics; Simulation; Tinkering; Integration; Genetic architecture; Branching; Teeth; Cancer; Appendages; Wing; Innovation; Mechanics

Abbreviations
Bmp bone morphogenetic protein
Dca Drosophila cold acclimation gene
Dpp decapentaplegic
ECM extracellular matrix
Eda ectodysplasin A
Edar ectodysplasin A receptor
Egf epithelial growth factor
Egfr epithelial growth factor receptor
EMT epithelial–mesenchymal transition
Fgf fibroblast growth factor
Gdnf/Ret glial cell line-derived neurotrophic factor/rearranged during transfection
Hgf hepatocyte growth factor
Hox homeotic box
Jak/Stat Janus kinase/signal transducers and activators of transcription
MCF-10A Michigan Cancer Foundation (human mammary gland epithelium)
MDCK Madin–Darby canine kidney
MET mesenchymal–epithelial transition
NF-κB nuclear factor kappa-light-chain-enhancer of activated B cells
PTEN phosphatase and tensin
QTL quantitative trait locus
Shh sonic hedgehog
Spry Sprouty
Tgf transforming growth factor
Tnf tumor necrosis factor
Upd unpaired
Wnt contraction of Wingless and Int (Integration site)

1. Introduction

Embryonic development and phenotypic diversification are closely linked phenomena. They have been fascinating scientists from different working fields for more than a century. After nearly half a century of complete separation between embryological and evolutionary studies, the two fields converged in the late 1970s–early 1980s, giving rise to the “EvoDevo” research field (Alberch, Gould, Oster, & Wake, 1979; Bonner, 1982; Gould, 1977; for reviews, see Amundson, 2005; Baguna, 2009). These pioneer Evo-Devo studies urged to uncover the generative rules of development, that is, the set of principles that connect all hierarchical scales of development together: molecules, cells, tissues, and organs. Traditionally, developmental studies were focusing on a few model organisms, whereas evolutionary studies were

examining taxa with high diversity and a good potential for preservation in the fossil record. Before genetic and developmental studies could be extended to nonmodel organisms, the inference of how development affected evolution was rather limited to theoretical predictions based on generic morphogenetic models (ie, Kauffman, 1993; Murray, 1989; Odell, Oster, Burnside, & Alberch, 1980; Oster, Odell, Burnside, & Alberch, 1979; Oster, Shubin, Murray, & Alberch, 1988). Since the beginning of this century, however, more and more experimental studies have attempted developmental comparisons of closely related species. Combined with mathematical models, these studies shed new light on how relatively conserved developmental pathways can drive phenotypic evolution.

Developmental systems—like other complex systems—exhibit an intriguing combination of robustness and capacity for change, whereby feedbacks between the levels of organization and context dependence are paramount. As tissue behaviors are hardly reducible to lower molecular levels, the atomist perspective is not satisfying (Urdy, 2012). Instead, modern developmental biology proposes a view that relies on nonlinear mechanochemical interactions. On the one hand, signaling molecules, organized into relatively conserved developmental pathways, modulate cell behaviors through nonlinear molecular interactions. On the other hand, cell behaviors such as proliferation, migration, and apoptosis necessarily generate mechanical stresses within the developing tissues (Ben Amar & Goriely, 2005; Dervaux & Ben Amar, 2008, 2011; Shraiman, 2005), and, as demonstrated by recent experiments both in vitro and in vivo, these mechanical stresses activate highly conserved mechanosensitive pathways (Aragona et al., 2013; Brouz# es & Farge, 2004; Dupont et al., 2011; Gieni & Hendzel, 2008; Ingber, 2006), which in turn modulate back cell behaviors (Chen, Mrksich, Huang, Whitesides, & Ingber, 1997; reviewed in Heisenberg & Bellai¨che, 2013). It is becoming clear that this framework is relevant not only for the morphogenesis of organs, their homeostasis, and renewal potential but also for their variation within populations and for their evolutionary diversification.

In this review, we will discuss in vitro, in vivo, and in silico studies on metazoan epithelial tissues to illustrate the conserved, dynamical, and complex aspects of their development. We will use as examples the insect wings, the vertebrate branched organs, and skin appendages like teeth, hair, and feathers. We will also use examples from epithelial cancer research, as it is becoming more and more recognized that cancer formation is a disease of development (Ingber, 2008) that also needs to be understood from an evolutionary perspective (Marusyk, Almendro, & Polyak, 2012). We will then explore the implications of the dynamical and nonlinear nature of development on the evolution of epithelial tissues at the phenotypic and genetic levels. Let us first provide some background information on epithelial tissues. Epithelial cells are ubiquitous in all metazoans. The tissues can be divided into three groups according to their embryonic origin (germ layers) in triploblastic metazoans: ectoderm, mesoderm, and endoderm. Both ectoderm and endoderm are usually organized as epithelial layers, while mesoderm is usually constituted of motile and contractile cells that secrete the major collagenous components of the mesenchyme (also known as stroma or connective tissue). Beneath virtually all epithelial sheets—even in some diploblastic metazoans such as sponges (Ereskovsky, Renard, & Borchiellini, 2013)—one finds a basement membrane composed of extracellular matrix (ECM) whose components include proteins organized into filaments (ie, collagen, laminin, fibronectin) and proteins linking these filaments together (ie, proteoglycans). In bilaterians, epithelial tissues line the surfaces of the body (skin), the glandular organs, as well as the internal cavities of the digestive, respiratory, and excretory systems (see review by Chuong, Wu, Plikus, Jiang, & Bruce Widelitz, 2006). In vertebrates, branched organs derived from the gut (lung, kidney, pancreas, and liver) are of endodermal origin (Fig. 1H), while organs derived from the skin are ectodermal in origin (Fig.1I). These ectodermal derivatives include various appendages such as scales, teeth, feathers, and hair but also glands (mammary gland, sweat gland, and salivary gland). In insects, ectodermal derivatives include all larval imaginal discs (wings, legs, and antenna). Epithelial tissues are at the interface with the outside world and as such act as essential barriers regulating the transport of molecules in and out of the body (Ashkenas, Muschler, & Bissell, 1996). Moreover, one estimates that 80–90% of cancers are epithelial in origin (carcinomas, data from U.S. National Cancer Institute’s Surveillance, Epidemiology, and End Results, http://training.seer.cancer.gov/disease/ categories/classification.html; see also Ingber, 2008). In addition, carcinogenesis requires genetic mutations and selective local microenvironments, the combination of which promotes somatic evolution (Gillies, Verduzco, & Gatenby, 2012). As such, comparisons between phenotypic evolution and somatic evolution of cancer are potentially highlighting. Thus, owing to their importance in development, evolution, and cancer, epithelial tissues and their derivatives are ideal systems to explore those interrelationships. Key to the development and function of metazoan epithelial tissues is

the establishment of apicobasal cell polarity—the differentiation of apical and basolateral cell membranes—and the organization of epithelial cells into simple, stratified, or pseudostratified layers. Carcinoma formation implies that epithelial cells undergo a partial epithelial–mesenchymal transition (EMT), that is, it requires a loss of epithelial cell polarity (Savagner, 2001) and a loss of cell–cell cohesion, leading cancer cells to migrate individually or collectively (Friedl & Wolf, 2010), invade the ECM, progressively disrupting tissue architecture. Epithelial cells are usually tightly packed and move in a collective way within the layer. This mechanical integrity stems from adherens junctions that hold neighboring cells together thanks to cadherins located in the lateral cell membranes (Knudsen & Wheelock, 2005). These cadherins also link to the cortical actin cytoskeleton inside the cells. Furthermore, integrins link the basal membrane and the cytoskeleton to the ECM.

The morphogenesis of most ectodermal derivatives in vertebrates (scales, teeth, feather, hair, and mammary gland) as well as the development of their glandular and branched endodermal organs requires a constant interaction between the epithelium and the underlying mesenchyme (Grobstein, 1953; Thesleff, Vaahtokari, & Partanen, 1995). Normal epithelial morphogenesis and regeneration sometimes require EMT with a transient reduction of polarity and adhesion (Ewald et al., 2012). These interactions and transitions are regulated not only via a reciprocal chemical signaling between the epithelial cells and the mesenchymal cells but also via mechanical interactions. Any protein interacting directly with a deformable structure—such as the ECM, the cytoskeleton, the nuclear membrane, and the nuclear chromatin—is a potential mechanosensor which—through conformation change—can alter the transcription of DNA in response to mechanical deformation (del Rio et al., 2009; Farge, 2011; Hynes, 2009; Johnson, Tang, Carag, Speicher, & Discher, 2007). The formation of carcinomas seems to imply partial disruption of this mechanical communication (Ingber, 2008). Cancer does not appear to be caused by uncontrolled growth in the general meaning of increased rates of cell proliferation: malignancy rather results when cells grow at times and in locations where proliferation is normally suppressed, that is, when they become partially nonresponsive to the controls that spatially and temporally constrain growth within living tissues during development and homeostasis (Ingber, 2003, 2005, 2006, 2008). In other words, cancers, but also possibly malformations and evolutionary innovations, may occur when some transitions that occur during normal epithelial morphogenesis are reused in another context.

In a first section, we explore findings regarding the shaping of metazoan epithelial tissues during development with an emphasis on the mechanical regulation of cell behaviors mediated by the interaction between the cells and the ECM. We discuss experiments and theoretical models highlighting that epithelial morphogenesis exhibits out-of-equilibrium dynamics, that cell proliferation rate is regulated by cell mechanics, and that tissue geometry feedbacks on its own morphogenesis. In a second section, we discuss the evolutionary genetics perspective on the diversification of organs derived from epithelial tissues, pointing out that the scarcity of examples makes it difficult to draw specific rules on the genetic basis of their evolution. In a third part, we will use the evolution of the mammalian molar tooth as a case study, to highlight how the nature of development leads to covariation of characters, which in turn provides some structure to evolutionary changes. We will argue that some of this integration is predictable if we have a good experimental and theoretical understanding of morphogenesis as exemplified by studies on the molar tooth in mammals. We will stress that small mutations may have large phenotypic effects, but also that a lot of mutations may have no phenotypic effect at all. This apparent contradiction stems from the nonlinearity of regulatory gene networks and the robustness of the morphogenesis of epithelial tissues. For the same reasons, we expect such nonlinear systems to be prone to genetic drift and cryptic genetic variation, and we will stress the context-dependence of mutational effects. We will also see that pleiotropic effects may favor the diversification of organs.

2. Shaping of epithelial tissues during development

2.1 On the Evolutionary Conservation of Molecular Pathways

Most organs in bilaterians build themselves out of simple epithelial sheets and mesenchymal cell masses. Since the 1980s, research has elucidated many of the genes that regulate cell division, cell polarization, cell migration, and cell differentiation. Most of those genes belong to evolutionary conserved protein families, some shared by all eukaryotes and others shared by all metazoans. Indeed, an ancient genetic toolkit which dates back to the split between choanoflagellates and metazoans (Erwin, 2009)—a

split corresponding to the origin of metazoan multicellularity—expanded and diversified through gene duplications. This ancient toolkit included transcription factors, signaling pathways, ECM, and adhesion molecules. The genetic diversification of this toolkit accompanied the extensive increase in the number of cell types and associated morphological diversification seen notably in bilaterians. Gene regulatory networks were also rewired through the evolution of cis-regulatory sequences and various noncoding RNAs controlling expression (Berezikov, 2011; Peter & Davidson, 2011). Note, however, that cell biology textbooks usually focus on intracellular structures and pathways perceived to be common to all cells, only occasionally addressing specializations in individual phylogenetic lineages. So the view that these structures are essentially invariant in diverse organisms engenders the false impression that they did not diversify much (Lynch et al., 2014). In present-day animals, these modified toolkits are used again and again during the morphogenesis of different organs within individuals, and across large parts of the phylogenetic tree, in organs that are or are not homologous (Carroll, Grenier, & Weatherbee, 2001). There are five major pathways present in bilaterians that are also present in cnidarians: Wnt, Tgf/Bmp, Notch, Hedgehog, and Jak/Stat (Janus kinase/signal transducers and activators of transcription) (see Erwin, 2009). In addition, the Fgf (fibroblast growth factor) and Egf (epithelial growth factor) pathways play an important role in bilaterian morphogenesis (Hogan, 1999). These pathways enable cell–cell communication through the interaction of a (secreted) ligand with its receptor, which often regulates transcriptional targets, including feedback regulators. On top of that are signaling pathways linked to cell–ECM adhesion and mechanosensitive pathways that emerged more recently in the literature as critical regulators of morphogenesis. Emblematic examples are, respectively, the integrins (first thought to be cell adhesion molecules) and the Hippo pathway, whose main components even predate the origin of metazoan multicellularity (Sebé-Pedrós, Zheng, Ruiz-Trillo, & Pan, 2012). Yorkie—or its vertebrate orthologs YAP and TAZ—is the nuclear effector of the Hippo pathway: it is a transcription coactivator playing critical roles in organ growth and cancer (Pan, 2010; Zeng & Hong, 2008; Zhou, Zhu, Xu, & Zhang, 2011). The Hippo pathway links physical parameters like cell density to determinants of cell polarity, to polarization of the actin cytoskeleton, and to cell proliferation in Drosophila and mammals (Amândio, Gaspar, Whited, & Janody, 2014; Aragona et al., 2013; Dupont et al., 2011; Fernandez et al., 2011; Fletcher et al., 2015; Lucas et al., 2013).

2.2 The Integrative Role of the ECM and Tissue Geometric Feedback

Although diverse, the major developmental pathways and material components thus appear relatively conserved. It appears also that limited sets of cell behaviors, combined into “morphogenetic routines,” are used repeatedly in different organs during development, regeneration, evolution, and cancer formation. This set of conserved routines (Chuong et al., 2006) are invagination, placode formation, bud formation, tube formation, condensation, branching, and folding (Fig. 1H and I). These transformations are typical of the epithelial–mesenchymal interaction, and they are calling for an ancient set of signaling pathways that regulates cell properties and their mechanochemical interactions. Although the critical molecular players have been identified, the rules of the game still need to be clarified. In particular, it is not clear how alterations in the regulation of signaling pathways affect the size and shape of organs. Below, we argue that we need to investigate in detail what happens at the cellular level if we are to progress on that question. One main difficulty at the cellular level lies in the fact that the inside and the outside of the cell are equally important in the regulation of cell behaviors. Below, we illustrate the integrative role that the extracellular environment is playing during morphogenesis and disease.

2.2.1 The Integrative Role of the ECM

Epithelial cells are asymmetric per definition because the secretion of ECM occurs on only one side, which thus becomes their basal side. The opposite, apical side usually faces either the outside world or a lumen, that also corresponds to the outside of the organism topologically. Epithelial cells indeed act as a physical barrier between the inside and the outside of an organism, regulating the chemical exchanges in and out, as for instance the absorption of nutrients and the secretion of wastes (Ashkenas et al., 1996). To achieve a particular tissue organization during development—in the form of sheets, tubes, or branches—epithelial cells have only a limited number of behaviors at their disposal: besides secreting and transporting various compounds, cells can proliferate, die, or migrate. Epithelial cells can also deform actively or passively, they can grow in volume, they can differentiate into a new cell type, and they can polarize. Yet,

the net effect of these behaviors depends, sometimes strongly, on their interaction with the ECM. Indeed epithelial cells are not isolated but packed against each other on top of the ECM so their behaviors are constrained by both their neighbors and the ECM via adhesion. Thus, epithelial cells can push or pull on their neighbors and the ECM, and they can induce changes into their neighbors and their microenvironment via the secretion of chemicals or via direct mechanical interaction. For instance, by pulling on the ECM, cells usually make the ECM stiffer as the ECM fibers align according to stress (see Van Oers, Rens, LaValley, Reinhart-King, & Merks, 2014 for a cell-based implementation of in vitro angiogenesis). We will see in the following that interactions between epithelial cells and the ECM can have conspicuous effects on the development at the cell and tissue level. In particular we have mentioned earlier that during normal development and also during cancer formation cells can convert between epithelial and mesenchymal phenotypes and vice versa (Ashkenas et al., 1996). For instance, neural crest cells, which are ectodermal derivatives, undergo an EMT, while they migrate along the anteroposterior axis of vertebrates in the process of head–face formation. Conversely, during kidney development in vertebrates, the process of nephron formation implies that mesenchymal cells of the metanephric mesenchyme undergo a mesenchymal to epithelial transition (MET). Again, as will become clear below, the modalities of these transitions are profoundly dependent on the cell–ECM interaction.

It has long been proposed that the interaction between the cells and the substrate or the ECM plays a critical role in controlling cell behaviors (Bissell, Hall, & Parry, 1982), most notably the establishment of cell polarity, the control of cell proliferation and migration, as well as the regulation of gene expression and cell differentiation. The earliest evidences came while setting up appropriate cell culture conditions. For instance, Madin–Darby canine kidney (MDCK) cells cultured in 2D on either plastic substrates (Fig. 1B) or permeable filters (Fig. 1C) exhibit size and differentiation differences: cells are typically about 3–5 mm tall when grown on solid supports, but about 10–15 mm tall when grown on filters (reviewed in Zegers, O'Brien, Yu, Datta, & Mostov, 2003). As epithelial cells receive most of their nutrients from the basal side (where, in vivo, the blood supply would be), culturing cells on solid substrates is indeed far from optimal. Consequently, cells are usually flat, poorly differentiated, and loosely polarized on solid substrates (Fig. 1B). When grown in 2D on permeable filters (Fig. 1C), cells can obtain nutrients through their basolateral surfaces, improving their growth and differentiation.

The addition of ECM components to in vitro cultures (Fig. 1D) participates in the regulation of gene expression, simulating in vivo-like cell behaviors. Without laminin (an ECM component found in vivo and in commercial Matrigel), mouse mammary cells that cannot synthesize their own ECM grow as a monolayer and fail to induce β-casein, regardless of the presence of lactogenic hormones (Bissell & Aggeler, 1987; Li et al., 1987). On the other hand, when cultured in the presence of lactogenic hormones and soluble laminin, these cells organize into three-dimensional clusters and express β-casein and other milk proteins at a high level. In fact, these cells are responsive to hormones and are able to express milk proteins only after they reorganized the laminin around them (Roskelley, Desprez, & Bissell, 1994). This shows that the cell–matrix attachment generates mechanical signals at the basal cell membrane that are transmitted to the nucleus and induce changes in gene expression (mechanotransduction). Likewise, cultured human salivary gland epithelial cells express the saliva protein cystatin only in the presence of laminin when cultured in 3D (Hoffman, Kibbey, Letterio, & Kleinman, 1996).

When cultured in 3D in matrigel (Fig. 1E and F), MDCK cells, and many other established cell lines, self-organize into hollow spheres, called cysts, formed by a monolayer of polarized epithelial cells. Cysts are reminiscent of the alveoli, acini, and follicles found at the end of tubules in many organs, such as lung, pancreas, mammary, and salivary gland (Fig. 1A; Zegers et al., 2003). Thus, 3D culture systems generally allow epithelial cells to organize into structures that resemble their in vivo architecture (Schmeichel & Bissell, 2003). Standard molecular and cell biology tools (ie, antibody inhibition, cDNA overexpression, RNA interference, and high-resolution imaging) can be applied to these organotypic cultures, making them a powerful and high-throughput system for deciphering the molecular and cellular aspects of epithelial morphogenesis in a biologically relevant context.

As more "in vivo-like" conditions are reproduced in 3D cultures than in 2D cultures, especially in matrigel (see reviews in Debnath & Brugge, 2005; Pampaloni, Reynaud, & Stelzer, 2007), organoids have been for instance instrumental to discovering the basic mechanisms by which cells polarize and form lumen (rewieved in Bryant & Mostov, 2008; Bryant et al., 2010; Datta, Bryant, & Mostov, 2011; Martin-Belmonte & Mostov, 2008; O'Brien, Zegers, & Mostov, 2002; Roignot, Peng, & Mostov, 2013). In mammals, apicobasal cellular polarization is evidenced by three polarity protein complexes—Par, Crumble, and

Scribble—which localize to different parts of the membrane of epithelial cells. The localization of these polarity complexes depends on cell–ECM. Notably, the disruption of these polarity protein complexes has marked effects on cellular proliferation, revealing that these complexes have key roles in tumor suppression (see review in Bryant & Mostov, 2008). Similarly, in Drosophila melanogaster, disruptions of ortholog genes that control cell polarity lead to hyperproliferation in vivo, suggesting again that the regulation of epithelial polarity, via cell–cell and cell–matrix interactions, may directly influence tumor progression (Bilder, Li, & Perrimon, 2000; Grifoni, Froldi, & Pession, 2013).

The culture of organoids in 3D has also clarified the critical role played by the ECM in controlling epithelial cell migration. It looks as if the physical resistance of matrix components enveloping organoids in 3D profoundly inhibits cell migration, regardless of the presence of factors that appear promigratory in other contexts. For example, treatment of MCF-10A cysts with Egf does not induce cell movement nor disrupt the organoid structure, whereas in 2D monolayers, the same treatment triggers a strong but nonoriented migratory response (Debnath & Brugge, 2005). Similarly, hepatocyte growth factor (Hgf) treatment is known to trigger loss of cell adhesion in 2D cultures but induces tubular branches of normal adherent cells in 3D cultures of MDCK cells (Fig. 1G; Kwon, Nedvetsky, & Mostov, 2011; Pollack, Runyan, & Mostov, 1998; Zegers, 2014; Zegers et al., 2003). The expression of matrix metalloproteinases in MDCK cells elicits the digestion of the ECM around the cells. This proteolysis in turn releases the physical constraints that the ECM was previously exerting on the cells, allowing them to proliferate and migrate. Such epithelial cell migration is required in most branching systems and cells usually transiently lose their apicobasal polarization and undergo a partial EMT (Ewald et al., 2012). Moreover, in vitro studies in kidney and salivary gland have revealed that epithelial cells exhibit high levels of motility and extensive cell rearrangements during branching morphogenesis (Chi et al., 2009; Ewald, Brenot, Duong, Chan, & Werb, 2008; Ewald et al., 2012; Larsen, Wei, & Yamada, 2006).

In summary, the cellular response to soluble factors, in particular whether this response recapitulates a normal morphogenetic event such as branching, or whether it reproduces an invasive behavior such as those seen in carcinomas, strongly depends on cell–ECM interactions that regulate cell polarity, proliferation, and migration. The ECM is actively remodeled by cells during development, normal tissue homeostasis, and repair (wound healing, inflammation). Notably, the ECM also interacts with the formation of gradients, for instance, by restricting the diffusion of ligands. As a consequence, the developing epithelia depends on both chemical and mechanical outputs. Such systems have been classified as morphodynamic (Salazar-Ciudad, Jernvall, & Newman, 2003), in the sense that at any step, the developing structure does not depend only on signaling but on other factors as well, in particular geometrical feedbacks that can influence diffusion in a nonlinear fashion (see also Urdy, 2012).

2.2.2 Mechanotransduction and Tissue Geometric Feedbacks

Likewise, recent experimental evidence demonstrates that mechanical stress generated during morphogenesis, via for instance cell proliferation or migration, activates mechanotransduction pathways, which in turn regulate cytoskeleton remodeling, cell proliferation, migration, and differentiation (Wozniak & Chen, 2009). Specifically, cultured stem cells differentiate as a function of the stiffness of their substrate: they differentiate into neurons on soft substrates and into myoblasts and osteoblasts on more rigid substrates (Engler, Sen, Sweeney, & Discher, 2006). This suggests that, in addition to morphogen concentrations, stem cell differentiation is under the control of the ECM stiffness (Discher, Janmey, & Wang, 2005; Discher, Mooney, & Zandstra, 2009).

The first experimental evidences of the role of mechanical stresses in regulating cell proliferation came from in vitro studies using microscalepatterned substrates coated with ECM components (Chen et al., 1997; Nelson et al., 2005). These studies showed that endothelial cells cultured on differently sized and shaped fibronectin islands behaved differently. Chen et al. (1997) showed that DNA synthesis, as a proxy for cell proliferation, scaled directly with projected cell area and not with cell–ECM contact area. Apoptosis was switched off by cell spreading, even though the cell– ECM contact area remained constant. Thus, cell shape per se appears to be the critical determinant that switches cells between proliferation, quiescence, and death. This shape-dependent regulation is proposed to act via the linking of integrins (and hence ECM) to the actomyosin cytoskeleton that transmit forces at the cell membrane to other locations within the cell and to the nucleus, where transcriptional events are triggered (Ingber, 2003, 2005, 2006, 2008). Nelson et al. (2005) also show that the positions of highest tensional stress correspond to sites of increased proliferation in vitro. For instance, on a circular island, the highest proliferation rates are observed at the periphery, but

on a square island they are observed at the corners. Inhibiting myosin-generated tension experimentally, or interfering with cell–cell adhesion, disrupts the transmission of mechanical tension and completely abolishes the growth differential. These studies demonstrate that tissue geometry can regulate proliferation.

Similarly, Nelson, Vanduijn, Inman, Fletcher, and Bissell (2006) show that tissue geometry also regulates cell migration; in particular, there is a correlation between the regions of high curvature and the positions of branches in mammary gland tubules grown on micropatterned islands. This correlation can be accounted for by a simple diffusion model in 3D. Let us assume that a molecule is secreted at a uniform rate by the epithelial cells and this molecule diffuses isotropically in the ECM. The geometry of the tissue will interfere with the diffusion; in particular the concentration of the molecule will be higher in flat regions. If this molecule inhibits the migration of cells in a dose-dependent manner, the regions of high curvature, where the concentration is lowest, must exhibit a relative enhancement of migration. Numerical simulations of this autocrine inhibitor system show that lowest concentrations of inhibitors occur at positions where branching is experimentally induced, specifically in regions of high curvature. These results were historically motivated by cancer research: during malignant transformation, progressive loss of cell shape-dependent growth regulation may lead to cell survival in the absence of cell spreading, leading to unrestricted mass expansion, and hence neoplastic disorganization of tissue architecture. Importantly, gradients of stress are determined by the geometry and size of the tissue, suggesting that the higher-ordered architectures (ie, iterative branching) of mature organs arise from mechanical feedback mechanisms that encourage the evolution of ever more morphologically complex structures from simpler ones. In conclusion, these studies provide a compelling evidence for the role of the stromal compartment and ECM in controlling epithelial cell function and dysfunction, and hence cancer growth, invasion, and metastasis. Theoretical cell-based models that incorporate a cell shape parameter (a measure of mechanical deformation and hence a good proxy for mechanical stress) are likely to shed light on the relationship between proliferation rate and mechanical stress.

2.3 Epithelial Topology: An Indication of the Out-of-Equilibrium Dynamics

In a monolayer epithelial sheet, cells, when viewed from their apical side, tend to have a polygonal shape as they tend to minimize the surface of contact with their neighbors (Fig. 2A). In a growing epithelial tissue, cells have different numbers of direct neighbors, that is, different numbers of polygonal sides. The distribution of numbers of cell sides is not random and indeed largely conserved among Metazoa (Gibson, Patel, Nagpal, & Perrimon, 2006). These authors propose that this statistical distribution (Fig. 2B) is a direct mathematical consequence of cell proliferation that can alter the number of sides of daughter cells as well as their neighbors (Fig. 2A): upon mitosis, a mother cell can divide into two daughters of lesser sidedness, while some neighbors can gain one side. In growing monolayer epithelia, the average number of sides is 6, but as cells have asynchronous but uniform cell cycle times, there is a significant proportion of cells with 4, 5, 7, 8, and 9 sides. Theoretically, uniform cell division can lead to the convergence of epithelial topology to a fixed distribution of numbers of cell sides. This theoretical distribution is in agreement with the experimentally observed distribution (Gibson et al., 2006). However, the model predicts that each cell in the population will gain an average of one side per cycle due to neighbor divisions (Fig. 2A; Gibson et al., 2006), so the distribution of numbers ofneighbors of (pre)mitotic cells only is expected to be shifted up: in average, mitotic cells indeed have seven neighbors in experiments (Fig. 2C; Gibson et al., 2006).

Numerical cell-based models (as opposed to continuous models) enable to explore the origins of these patterns by testing quantitatively alternative scenarios. For instance, Aegerter-Wilmsen et al. (2010) show that the destabilizing effects of proliferation cannot account for the neighbor distribution of mitotic cells (Fig. 2C) and that this conserved topology most probably emerges from the mechanical properties of epithelial cells and the way they interact with their neighbors. By testing several theoretical scenarios that differed in whether cell proliferation is dependent on mechanical stress or not, they show that only the scenarios that assume mechanical stressdependent proliferation are in agreement with experimental data. Indeed a cell with a low number of sides (ie, five neighbors) tends to be compressed by its neighbors, whereas a cell with a high number of sides (ie, eight neighbors) tends to be stretched by its neighbors. Thus, if cell proliferation rate is set to be proportional to the number of direct neighbors, and thus to apical cell area, the local mechanical stresses within the tissue tend to be released and the numerical simulations reproduce the two observed experimental distributions of cell sides (Fig. 2B and C). Such a regulation seems favorable for maintaining the integrity of tissue structure during growth, by preventing cell crowding

and extrusion of healthy cells off the tissue. Indeed, spatial differences in cell proliferation rate lead to the accumulation of local stresses that cannot be relieved by an extensive rearrangement of cells, especially in epithelia where the timescale of cellular rearrangements is larger than that of proliferation. For instance, a clone of rapidly proliferating cells surrounded by a clone of slowly proliferating cells is under compression (Shraiman, 2005). If the growth rate of the cells in the rapidly proliferating clone is not inhibited, these cells will grow on top of each other, disrupting the architecture of the original monolayer in a way similar to the way tumors grow. However, this does not happen in healthy tissues: experiments show instead that the proliferation rates in the different clones rapidly converge, allowing homeostasis of the monolayer architecture (Shraiman, 2005). Therefore, if one assumes that there exists a mechanical feedback on proliferation rate—where the proliferation rate of compressed cells is down-regulated—one can predict that growth rates within the tissues will converge. This largely uniform distribution of growth rates appears as a mechanism preventing the progressive accumulation of stresses within the tissues, a process that favors homeostasis.

In addition to the regulation of proliferation rates, the orientation of mitoses needs to be regulated too. Countless studies of cell division in 3D structures showed that miss-oriented cell mitoses cause the formation of multiple lumen. 3D cell cultures are ideal to elucidate how the orientation of cell divisions is regulated. In order to clarify the role of mitotic plane orientation in the emergence of multilumen phenotypes, a recent study investigated the distribution of the number of cell sides in developing MDCK cysts in 3D culture (Cerruti et al., 2013). These authors observe that the distribution of the number of cell sides in normal MDCK cysts is similar to that found by Gibson et al. (2006). Devising a numerical cell-based model of cystogenesis—including apicobasal polarization, de novo lumen formation, and proliferation—Cerruti et al. (2013) show that the distribution of Gibson et al. (2006) is generated in silico at the condition that cell proliferation is faster than mechanical relaxation, that is, if the timescale at which proliferation occurs is significantly smaller than the timescale at which cell rearrangements occur. This condition, with two different timescales, defines out-of-equilibrium dynamics. Cerruti et al.'s cell-based model is further useful to understand the dynamics at work during cystogenesis and cancer formation, as it predicts that an equilibrium distribution will be attained instead of the out-of-equilibrium one, if the two timescales converge. This convergence can be achieved by significantly decreasing the rate of cell proliferation, by altering cortical contractility, cell–cell or cell–ECM interactions, or by increasing cell motility. Cerruti et al. (2013) further test these predictions experimentally. By releasing cortical tension, they confirm that the distribution of the number of cell sides converges toward the predicted equilibrium distribution (in this spherical case, mainly hexagons with a few pentagons, like the patches on a soccer ball). They further show that disruption of mitotic plane orientation results in aberrant multiluminal cysts, but only in conjunction with out-of-equilibrium dynamics. Indeed, slowing down cell division partially rescues the multilumen phenotype induced by miss-oriented cell division planes. This rescue thus confirms that if the rate of cell proliferation is decreased sufficiently, miss-oriented cell divisions are not sufficient to induce a multilumen phenotype. This model suggests however that an increase in proliferation rates, a typical hallmark of cancer, could alone be responsible for the appearance of aberrant multilumen phenotypes, independently of the control of mitotic plane orientations. Apparently, even if a strict control of cell polarity ensures the optimal orientation of mitoses, multilumen phenotypes will nevertheless occur if cell proliferation is much faster than cell rearrangements. Indeed, the incidence of single lumen cysts in normal MDCK cells in 3D culture is only 50% after 2 days of culture and it reaches 80% after 5 days of culture (Cerruti et al., 2013). Thus, the proportion of multilumen cysts naturally decreases when growth rates decrease. Small lumen tend to fuse with larger lumen by sliding along the lateral cell–cell interfaces. Thus, lumen coalescence does not appear to necessitate cell–cell rearrangements (Cerruti et al., 2013). However, the process of lumen coalescence is still slow compared to normal proliferation. A recent study (Bosveld et al., 2016) shows also that the orientation of cell divisions in the Drosophila pupal notum is governed by the distribution of tricellular junctions, hence the number and location of neighboring cells. This study combines experimental and numerical cell-based simulations to illustrate that the topology of the tissue is used as a mechanical and polarity cue to orient the mitotic spindles. Interestingly, these junctions are known to be the sites of enrichment of several proteins including adhesion molecules, cytoskeleton regulators, and Hippo pathway components (Furuse, Izumi, Oda, Higashi, & Iwamoto, 2014; Lye, Naylor, & Sanson, 2014; Oda, Otani, Ikenouchi, & Furuse, 2014; Rauskolb, Pan, Reddy, Oh, & Irvine, 2011; Sawyer, Harris, Slep, Gaul, & Peifer, 2009).

In the next section, we discuss two in silico cell-based models that proposed a mechanical regulation

of cell proliferation to account for growth arrest in the imaginal wing disc of D. melanogaster.

2.4 Regulation of the Drosophila Wing Disc Growth: Insights into Cell Mechanics

The Drosophila imaginal wing disc is an epithelial sac with a central lumen. The epithelial layer on the dorsal side of the larva is called peripodial membrane, while the layer on the ventral side is called the disc proper. Most of the recent studies on the regulation of organ size focus on the growth of the disc proper. Several authors proposed models, where growth rates depend both on morphogen concentration and on the mechanical stresses that naturally arose from the nonhomogeneous spatial distribution of the morphogen that stimulated growth in the first place. Hufnagel, Teleman, Rouault, Cohen, and Shraiman (2007) assume that cells proliferate when the concentration of decapentaplegic (Dpp) exceeds a given threshold. Cells stop proliferating if they are mechanically compressed and the Dpp concentration falls below this threshold. Similarly, Aegerter-Wilmsen, Aegerter, Hafen, and Basler (2007) propose a model where growth is stimulated by two morphogen gradients, the maximal concentration of both morphogens occurring at the center of the disc. As the center grows, the peripheral regions undergo tangential stress, which stimulates their growth. Since the stretching is not completely compensated for by the growth of peripheral cells, the center is compressed in return and its growth is inhibited.

In these two models, the distribution of mitoses (hence growth) tends to be uniform across the disc after some time, which is in agreement with in vivo observations (Mao et al., 2013; Milàn, Campuzano, & Garcìa-Bellido, 1996). As predicted by these models, the cell compression gradient is maximal at the center of the disc in vivo, and this gradient increases with age (Nienhaus, Aegerter-Wilmsen, & Aegerter, 2009). In other words, the apparent paradox that proliferation is uniform while the growth promoter Dpp is not distributed uniformly disappears if one takes into account the mechanical stresses generated during growth. Additionally, it provides a scheme to understand how the balance of chemical and mechanical factors can lead to growth arrest. In the next section, we turn to branching morphogenesis and illustrate the conservation of signaling pathways and “morphogenetic routines” in various branched organs. We discuss also how the tissue geometry can be understood as an actor of its own morphogenesis, and thus its own shape, which suggests that branching is a morphodynamic process.

2.5 Branching Morphogenesis: A Mirror of Diffusion-Limited Growth

Epithelial tissues form highly branched tubular structures in many secretory organs. The tubes themselves are made of monolayers of polarized epithelial cells facing a central lumen. In mammals, examples include the mammary gland, the prostate, the pancreas, or the salivary glands. Branched tubular structures are also found extensively in organs whose main function is to transport and distribute gases or liquids: for example, in lungs, kidneys, and blood vessels. A branched tubular structure is usually built by the iterative use of a few simple “morphogenetic routines”: bud initiation, bud extension, and bud splitting at its end (Metzger & Krasnow, 1999). For instance, in the mammalian lung, beginning on embryonic day 9 in mouse (about day 25 in humans), two epithelial buds sprout from the foregut into the surrounding mesenchyme to form the left and right primary bronchi. The primary bronchi grow and sprout secondary bronchi, which sprout tertiary bronchi, and so on. Branching continues for a total of 6–8 generations in the mouse and for about 20 generations in humans (Metzger & Krasnow, 1999).

2.5.1 Molecules Involved in Branching Morphogenesis

Branching in general requires a mesenchymal–epithelial cell–cell interaction mediated by an Fgf in mammals, and its ortholog (branchless) in Drosophila. Usually, the mesenchyme greatly influences the pattern of branching. One striking example comes from lung–kidney tissue recombination experiments in which lung mesenchyme induces the kidney epithelium (ureteric bud) to form branches with a pattern characteristic of lung epithelium (ie, increased lateral branching) (Lin et al., 2001). The molecular players involved in branching are particularly well known in the mammalian lung (Cardoso, 2006; Miura, 2015) and relatively well known in the kidney (Blake & Rosenblum, 2014; Costantini & Kopan, 2010). The three most important molecules involved in lung branching—as evidenced by the number of citations in Pubmed (Miura, 2015)—are fibroblast growth factor 10 (Fgf10), sonic hedgehog (Shh), and bone morphogenetic proteins 4 (Bmp4). There is also evidence that activation of Fgf10 signaling in the developing pancreas, tooth, skin, and lung may be required to expand or maintain a pool of epithelial progenitor cells during

organogenesis (Bhushan et al., 2001; Harada, Mitsuyasu, Toyono, & Toyoshima, 2002; Norgaard, Jensen, & Jensen, 2003). Components of the Fgf signaling pathway are detected in the ureteric bud and metanephric mesenchyme, the two progenitor tissues that interact through reciprocal signaling in the developing kidney (Qiao et al., 2001, 1999). Most of the experimental studies devoted to branching morphogenesis repeatedly listed the molecules which were required for branching. However, how these molecules affect cell behaviors like cell proliferation and cell migration is still poorly understood. In the mammalian lung, bud initiation starts with the mesenchyme secreting Fgf10 at some stereotyped locations along the foregut (Bellusci, Grindley, Emoto, Itoh, & Hogan, 1997; Sekine et al., 1999). The epithelium secretes positive and negative regulators of Fgf10, in particular Shh which counteracts Fgf10 expression (Bellusci, Furuta, et al., 1997; Lebeche, Malpel, & Cardoso, 1999; Pepicelli, Lewis, & McMahon, 1998). It is thought that Fgf10 expression is graded, with the highest expression being located at the distal end of the mesenchyme (Bellusci, Grindley, et al., 1997). In response to Fgf10, epithelial cells initiate a bud that extends as a stalk into the mesenchyme toward the Fgf10 source. This extension is driven by proliferation (Bellusci, Grindley, et al., 1997) and/or chemotaxis (Park, Miranda, Lebeche, Hashimoto, & Cardoso, 1998; Weaver, Dunn, & Hogan, 2000). Fgf10 reception at the tips induces the expression of secondary genes in the epithelium, such as Bmp4 and Sprouty2 (Spry2). Bmp4, expressed in the distal epithelium and proximal mesenchyme, is thought to inhibit Fgf10 signaling and to limit epithelial cell proliferation (Bellusci, Henderson, Winnier, Oikawa, & Hogan, 1996; Weaver et al., 2000). Spry2, expressed at the tips of the epithelial buds, inhibits Fgf10 signaling and limits the proliferation or migration of the lung epithelium when buds are forming (Mailleux et al., 2001). Hence, both Bmp4 and Spry2 constrain growth to occur further away from the tips, presumably leading to the branching. Shh has also been implicated in the regulation of mesenchymal cell proliferation (Weaver, Batts, & Hogan, 2003).

The situation is remarkably similar in the Drosophila trachea (Metzger & Krasnow, 1999; Park et al., 1998), with the reiterative use of the Fgf signaling pathway and similar organizational features: Branchless expression—an ortholog of Fgf—initiates buds (called epithelial sacs) at 20 locations along the embryo and turns theses sacs into primary branches by regulating cell migration (Sutherland, Samakovlis, & Krasnow, 1996). Sprouty expression—an ortholog of Sprouty2 (Tefft et al., 1999)—inhibits Branchless expression and restricts branch splitting to the tips (Hacohen, Kramer, Sutherland, Hiromi, & Krasnow, 1998). Notably, although cell proliferation is involved in the branching of vertebrate lungs, only cell migration is used in the branching of the Drosophila trachea. Other molecules have been implicated in the control of lung branching morphogenesis in mice, in particular members of the Tgf-β subfamily. These proteins, secreted in the mesenchyme, tend to accumulate along the stalks and in between buds (Heine, Munoz, Flanders, Roberts, & Sporn, 1990). They may also inhibit Fgf10 signaling and induce the secretion of ECM components, thus inhibiting budding (Lebeche et al., 1999; Tomlinson, Grindley, & Thomson, 2004). In Drosophila, integrity and proper sulfation of heparan—one of the most abundant component of ECM—are essential for mediating Fgf signaling and tracheal morphogenesis (Kamimura et al., 2001).

The role of Fgf signaling in branching morphogenesis of the mammalian kidneys is not as well known as in the lungs (reviewed in Blake & Rosenblum, 2014; Costantini & Kopan, 2010). Yet, Fgf7-null mutants have fewer branch points and ectopic Fgf7 in organ culture can stimulate branching (Qiao et al., 1999). In vitro, Fgf1 and Fgf10 affect elongation of the bud stalk before the branch-point decision is made (Qiao et al., 2001). By far the most intensely studied signaling pathway involved in renal branching morphogenesis is the glial cell line-derived neurotrophic factor/ rearranged during transfection (Gdnf/Ret) tyrosine kinase signaling pathway (reviewed in detail in Davis, Hoshi, & Jain, 2014). Gdnf—secreted in the mesenchyme—is a major inducer which controls the outgrowth of the ureteric bud with a chemotactic effect (Sariola & Saarma, 2003; Tang, Cai, Tsai, Wang, & Dressler, 2002; Tang, Worley, Sanicola, & Dressler, 1998). The Tgf-β signaling pathway is another important pathway that controls renal branching morphogenesis (reviewed in Nishinakamura & Sakaguchi, 2014). Bmp4 is expressed in several tissues of the developing kidney (Dudley & Robertson, 1997), is required for normal renal development (Dunn et al., 1997), and negatively regulates ureteric bud outgrowth and branching (Miyazaki, Oshima, Fogo, Hogan, & Ichikawa, 2000). The addition of exogenous Bmp2 in culture also inhibits renal branching morphogenesis (Fisher, Michael, Barnett, & Davies, 2001; Martinez, Mishina, & Bertram, 2002). Other secreted stromal components shown to regulate branching morphogenesis include components of the ECM which may play a vital yet relatively unknown role during renal branching morphogenesis. In summary, we need to understand how a wealth of molecules and signaling networks influence a set of cell behaviors in three dimensions, in a highly nonlinear, complex, and dynamic system. Theoretical modelers strive to provide a

framework that can make sense of the in vivo and in vitro experiments. Several theoretical models of branching have been proposed so far. Most of them focus on the critical role of Fgf in promoting bud outgrowth and chemotaxis. To date, only a few numerical cell-based models have attempted to simulate branching and we argue below that cell-based simulations would provide much insight into how slight modifications of signaling networks would finely tune cell behaviors, which in turn would account for organ-specific patterns of branching.

2.5.2 Theoretical Modeling of Branching Morphogenesis

Several theoretical models can generate branching patterns that mimic structural features of branched organs such as the self-avoiding branching pattern of the lungs. As discussed earlier, experimental studies have demonstrated the central role of Fgfs and Shh in growth and branching of epithelial tissues. However, the mechanism underlying the way branching events is organized at the organ scale can be fully appreciated only by building models. One of the first models of lung branching suggested that branching was analogous to viscous fingering (Lubkin & Murray, 1995), whereby two fluids of very different viscosity—the luminal fluid and the mesenchyme—are separated by a "skin" of surface tension, aka the epithelium. The viscosity of the luminal fluid is negligible, whereas the viscosity of the mesenchyme is several orders of magnitude higher. Note that viscous fingering is analogous to diffusion-limited aggregation with surface tension and exhibits similar tip-splitting patterns.

In 2002, Miura and Shiota proposed a reaction–diffusion model to simulate the in vitro culture of mesenchyme-free lungs, which branch in the presence of Fgf and Matrigel (Nogawa & Ito, 1995). In reaction–diffusion models, the spatiotemporal distributions of two or more substances are explained by the combination of two processes: local reactions during which the substances are antagonizing each other, and diffusion, which causes these substances to spread out in space. These models are often used to account for self-organized patterns such as stripes, ripples, washboard patterns, and dendrites, and various processes of morphogenesis. In Miura and Shiota's model, Fgf1 is assumed to promote growth of the epithelial cells in a concentrationdependent manner. Cells exposed to Fgf1 grow, but at the same time deplete Fgf1 locally, which inhibits further growth laterally. Because the initial shape of explants is not ideally spherical, some cells are necessarily closer to the source of Fgf1, initiating budding at random locations. As lateral growth is inhibited, the buds extend into stalks and grow even faster as they get closer to the Fgf1 source. This mechanism results in a "protrusion grows faster" tendency, and such positive feedback amplifies the subtle initial fluctuations of the explant's shape and produces a branched pattern. Such mechanism is also known as diffusion-limited growth or Laplacian growth. The Laplacian instability occurs when a smooth interface evolving under a Laplacian field (such as a concentration field) develops rapidly growing spikes and branches. This mechanism has been used to explain many disparate branching patterns (Fleury, Gouyet, & Leonetti, 2001), such as dendrites on snowflakes, lobes on leaves, vasculogenesis, and coral growth (Merks, Hoekstra, Kaandorp, & Sloot, 2003), among others.

To simulate the in vivo case, Hirashima, Iwasa, and Morishita (2009a) model Fgf10 signaling together with the diffusion of Shh and Tgf-β, confirming that a split-expression domain may emerge in a reaction–diffusion system with three interacting molecules. They further show that when the distance between the tip and the lung border is large, Fgf10 expression domain is single peaked, which can be interpreted as promoting branch elongation. When the distance between the tip and the lung border is small, Fgf10 expression shows two peaks whose locations depend on the curvature of the lung border. This split-expression domain is used to account for terminal bifurcation or lateral branching. Hirashima et al. (2009a) predict that lateral branching can hardly occur in low curvature geometries, such as the kidney, which is in agreement with experimental data: lateral branching is known to occur in lungs (Metzger, Klein, Martin, & Krasnow, 2008) but is not observed in kidneys (Short, Hodson, & Smyth, 2013).

Reaction–diffusion models are known to be really sensitive to noisy initial conditions, and the parameter space that reproduces experimental data is usually quite narrow. In order to investigate these issues, Menshykau, Blanc, Unal, Sapin, and Iber (2014) simulate an alternative reaction– diffusion model on experimentally obtained 3D embryonic lung domains (Blanc et al., 2012). This model is a ligand–receptor-based (LR) Turing mechanism. Four scenarios are investigated depending on whether the ligand is diffusing in the mesenchyme and the receptor is in the epithelium or vice versa and whether the ligand is an activator or an inhibitor of bud outgrowth. They find out that the predicted 3D expression domains are in agreement with the observed experimental growth field if the ligand is either a growth activator that diffuses in the mesenchyme or an inhibitor that diffuses in the epithelium. Interestingly, these simulations

yield both bifurcating and trifurcating branch points, both of which are known to occur in the lung (Metzger et al., 2008). The first scenario would correspond to an Fgf10-based LR Turing mechanism, whereas the second scenario would correspond to an Shh-based LR Turing mechanism. Both mechanisms are not mutually exclusive, and a network based on the couple Fgf10/ Shh (Shh negatively regulating Fgf10) enlarges the parameter space for which the embryonic data are satisfactorily reproduced. Menshykau et al. (2014) show that this LR Turing mechanism is less sensitive to noisy initial conditions than the simple Turing mechanism. Furthermore, they demonstrate that the expression of ligands and receptors in different tissue layers is critical for the emergence of a geometry effect that ensures robust pattern formation in spite of molecular noise. Menshykau et al. (2014) therefore conclude that the combination of geometry and signaling enables robust branching morphogenesis.

Few studies explicitly simulate cellular behaviors such as cell division and chemotaxis as driving forces of branching. In such an attempt, Hirashima, Iwasa, and Morishita (2009b) construct a cell-based model for ureteric bud morphogenesis, where two fixed sources of Gdnf are placed on each side of a tip. In this model, Gdnf works as both a growth factor and a chemoattractant, as suggested by experiments. Hirashima et al. (2009b) find that the resulting morphology follows three major patterns: abnormal kinked, bloated, or a normal bifurcating pattern, the major determinant of the emerging morphology being the balance between the rate of cell proliferation and the rate of cell migration by chemotaxis. If proliferation is low compared to migration, the tips become bloated, as in mutants treated by TGF-β (Davies, 2005). If migration is low relative to proliferation, the tips become kinked as in mutants where PTEN is activated (Kim & Dressler, 2007). This model then uncovers an issue that is often overlooked: the morphology is not expected to change as long as the balance between cell behaviors is maintained.

In most models, except purely mechanical ones (ie, Lubkin & Murray, 1995), a molecule assumes the role of a chemo-attractant and/or of a growth factor. Although these models differ in some of their assumptions, they share one principle accounting for branching: the location of initiating buds depends on a dynamic but reproducible interaction between multiple factors that act in concert in both the epithelium and the mesenchyme to establish a gradient of a molecule that promotes bud initiation. This rather simple mechanism is geometry driven and can generate the observed branching topologies. Conversely, these models suggest that the geometry of the developing organ strongly affects the spatial distribution of diffusive chemicals, a sensitivity characteristic of dynamic systems (Murray, 1989). In other words, branching morphogenesis is a morphodynamic process (Salazar-Ciudad, Jernvall, & Newman, 2003). Furthermore, these models show that specific regulation of each tip is not required for the emergence of striking geometrical features such as branching tubules. Now that a wealth of 3D information on the normal and pathological sequences of branching events is becoming available for kidneys and lungs (Combes et al., 2014; Lamberton, Lefevre, Short, Smyth, & Hamilton, 2015; Short et al., 2014, 2013) and that 3D growth fields of embryonic lung buds can be computed (Blanc et al., 2012; Menshykau et al., 2014), there remains to understand the finer details of the branching topologies such as the diameter of the branches, their bifurcating angle, and their rotation angles. Studies that incorporate cell behaviors such as proliferation, migration, apoptosis, cell–cell adhesion, and ECM remodeling are most likely to provide the necessary insights on how quantitative modifications in the genetic pathways underlying branching morphogenesis may affect phenotypic variation in branched organs during disease, regeneration, and evolution. Such simulations could even unveil strategies for reconstructing branched organs ex vivo. It may come as a surprise but, as we will see in the next section, tooth morphogenesis has much in common with branching morphogenesis.

2.6 Mammalian Tooth Morphogenesis: An Emblematic Illustration of Morphodynamic Development

Most of what we know about the development of teeth is based on mice experiments. Mice are an iconic model for developmental genetics and a large amount of data are now available on their dental development (see http://bite-it.helsinki.fi/ for a database; Jernvall & Thesleff, 2000, 2012). Teeth are initiated from the dental lamina, a stripe of stratified epithelium that forms at E11 and restricts the tooth-forming area laterally. At E12, placodes, which are local thickenings of the dental lamina, appear. At this stage, most of the expression of dental lamina genes becomes restricted to these placodes, or to just a small cluster of placodal cells called the early signaling center (Balic & Thesleff, 2015), whose signals drive the invagination of the placode and the formation of the tooth bud. At the tip of the bud, epithelial cells get packed and stop growing, forming the so-called primary enamel knot. Growth keeps going on the sides of

the enamel knot, giving rise to the cervical loops (cap stage). During the following bell stage, secondary enamel knots are formed in multicusped teeth (molars), which determine the positions and heights of the tooth cusps (Jernvall, Kettunen, Karavanova, Martin, & Thesleff, 1994). During this process, the same signaling pathways are used reiteratively (Jernvall & Thesleff, 2000). They are also used for tooth renewal (Jernvall & Thesleff, 2012). The same molecular signaling (in particular Fgf4, Shh, and Bmp4) is also involved in other skin appendages and, as we have seen previously, in the branching morphogenesis of mammal lungs and insect trachea (Metzger & Krasnow, 1999). While tooth cusp morphogenesis consists in adding reiteratively new secondary enamel knots within an existing tooth crown base, lung morphogenesis occurs through reiterative addition of new lung buds to the tips of existing branches. Lung bud tips have been indeed proposed to act as signaling centers (Metzger & Krasnow, 1999), in a manner that is analogous to that of the secondary enamel knots at the cusp tips in teeth (Jernvall & Thesleff, 2000).

Salazar-Ciudad and Jernvall (2002, 2010) have developed a theoretical vertex model at the cellular level that simulates the interactions between the epithelium and the underlying mesenchyme. As a starting point, the model includes a set of identical epithelial cells lying above a set of identical mesenchymal cells. All cells can respond to mechanical cues as well as to three diffusive signaling molecules: an activator inducing enamel knots, an enamel knot-secreted inhibitor of enamel knot formation, and a growth factor regulating growth of the epithelium and mesenchyme. Because the enamel knot differentiation is irreversible, the model represents an irreversible reaction–diffusion-like model. Contrary to a standard reaction– diffusion model, the 3D domain within which the signals diffuse grows as the reaction–diffusion patterning mechanism operates. As a consequence, the way the signals diffuse is constrained by the domain but the growth of the domain depends also on the diffused signals. In other words, pattern formation and morphogenesis are mutually linked, a mode of development, they, and we after them, call morphodynamic (Salazar-Ciudad & Jernvall, 2002). Established candidate molecules for activators include Bmps, which induce differentiation markers in the dental epithelia and are associated with proliferation arrest in the knot. Potential inhibitors include Fgfs and Shh, which stimulate growth and survival of dental epithelia, mesenchyme, or both, Fgfs being antagonist of Bmps (Salazar-Ciudad & Jernvall, 2002). The first cusp forms when epithelial cells differentiate into nondividing enamel knot cells, which happens when activator concentration reaches a set threshold. Knot cells secrete the inhibitor, which counteracts the secretion of activator, thus also inhibiting the formation of the second cusp immediately adjacent to the first cusp. Formation of the second cusp also depends on the geometry of cusps (their sharpness), because the geometry modifies the volume of mesenchymal tissue into which the molecules diffuse (Salazar-Ciudad & Jernvall, 2002). It is worth noting that, as for other organs, this model highlights that absolute concentrations of signaling molecules are likely to be less critical for the resulting phenotype than the ratios of say activators and inhibitors.

So far, we have discussed how epithelial tissues are shaped during development, and we have seen that the morphogenesis and homeostasis of epithelial tissues requires a constant, dynamical, and reciprocal interaction between the epithelial cells, the ECM, and the mesenchyme. These interactions involve both chemical and mechanical inputs which are integrated by the actomyosin cytoskeleton. That way, the changes occurring outside the cells can be transduced to the nuclei where transcriptional regulation occurs. As a consequence, cell behaviors are dependent not only on signaling networks but also on cell shape, a good proxy for mechanical stress. At the tissue level, one can view the tissue geometry as an actor of its own morphogenesis, in a morphodynamic sense. Changes in domain size and domain shape affect the distribution of diffusing chemicals as well as the distribution of mechanical stresses, which in turn modify organ's shape. Theoretical models are useful to investigate these puzzling effects and they contribute to drawing a general view of the basic principles underlying organ morphogenesis. Cell-based models and cell-based experiments, in particular organoid systems, appear necessary to connect the dots between the molecular and phenotypic levels. Given the relatively large conservation of signaling networks, of structural components, and of "morphogenetic routines," most differences in phenotypes can, in the end, be related to subtle spatial and temporal modifications in the deployment of cellular behaviors. Most importantly, theoretical cell-based models generally show that the absolute rate of processes is rarely important. What matters most is the ratio between the rates of different processes: the rate of cell proliferation relative to the rate of cellular rearrangements as exemplified in MDCK cysts; the rate of cell proliferation relative to the rate of cell chemotaxis as exemplified in branching morphogenesis; and the relative strength of activation compared to inhibition as exemplified in teeth morphogenesis. This cell-based approach, combining insightful in vitro experiments with insightful theoretical models, appears critical to understand diseases

like cancer formation, but also evolutionary innovations. In the next section, we discuss how the morphodynamic nature of morphogenesis may impact the way epithelial tissues can diversify.

3. The evolutionary genetics perspective on the diversification of organs derived from epithelial tissues

3.1 Innovations, Tinkering, and Genome Duplications

The origin of evolutionary innovations is one of the most fundamental questions of biology. Possibly the main conclusion of the last 20 years of research in evolutionary developmental biology is that most of the genetic circuitry that is involved in the development of biological structures is conserved across the animal kingdom (Carroll et al., 2001). At least at phylogenetic scales where causative changes can be identified, most innovations and most interspecific differences in animals rely not on new genes, but on mutations at homologous genes (Martin & Orgogozo, 2013) and, usually, on either spatial (heterotopies) or temporal (heterochronies) modifications of their expression domains. These modifications may indeed lead either to the coexpression of particular genes, conferring new physicochemical properties to the corresponding cells, or to the deployment of the same gene in a new microenvironment (cells or regions that display particular properties under particular constraints). This conservation of the main developmental modules and their cooption at new times and places reflect the fact that animals are complex dynamical systems originated from a common ancestor: as put nicely by Jacob (1977, p. 1164), evolution proceeds by tinkering, it “uses everything at its disposal to produce some kind of workable object. [..] Evolution does not produce novelties from scratch. It works on what already exists, either transforming a system to give it new functions or combining several systems to create a more elaborate one.”

Relatively rare duplications of particular genes or even duplication of the whole genome (Holland, Dehal, & Boore, 2005) may occasionally release some of the presumed strong constraints imposed by natural selection, thereby allowing more “acrobatic” tinkering: the duplicated, “unneeded” gene copies may evolve more easily, eventually acquiring new functions. For instance, homeotic box (Hox) genes were important for the diversification of body plans and the generation of new segment types in arthropods (Averof, 1997; Averof & Akam, 1995; Kmita & Duboule, 2003). Because the last common ancestor of cnidarians and bilaterians had only two Hox genes (Chourrout et al., 2006) and acoel flatworms (which may represent a good approximation of the ancestral bilaterian) possess only three Hox genes, whereas the protostome/deuterostome ancestor must have had at least seven different Hox genes (de Rosa et al., 1999), the different Hox genes of protostomes and deuterostomes are most likely the result of duplications followed by sequence divergence (de Rosa et al., 1999; Schubert, NieseltStruwe, & Gruss, 1993).

Yet, gene duplications per se do not explain innovations: for example, almost identical sets of Hox genes are found in arthropods with very different segmental patterns (Averof, 1997). Only the subsequent differentiation of the duplicated genes or modifications of their expressions may account for evolutionary novelties. A scenario, consistent with the evidence from the fossil record (Wilson & Caldwell, 1993), has been proposed (Coates & Cohn, 1998) for the origin of paired appendages in vertebrates, whereby the necessary independence of Hox gene regulation in paraxial and lateral plate mesoderm was achieved by divergence of initially similar Hox gene expressions in both tissues, possibly through gut regionalization and subsequent stabilization of the new Hox boundaries in the lateral plate mesoderm. Similarly, the Hox gene duplications underlying the diversity of Hox genes found in modern insects had already occurred before the divergence of insects and crustaceans, probably in the Cambrian, long before the trunk segment diversification in the lineage leading to insects took place (Akam et al., 1994). In that respect duplications are not fundamentally different from the process of cumulative mutations and they do not fully and convincingly explain major innovations. In fact, earlier hypotheses that gene duplication events correlated with apparent patterns of bursts in morphological complexity of vertebrates (Ruddle et al., 1994; Sidow, 1996; Stellwag, 1999) are not tenable in the light of the fossil record: the corresponding, apparent gaps between the living branches of the vertebrate tree are filled up by series of extinct but intermediate taxa (Donoghue & Purnell, 2005). In the next section, we focus more specifically on the evolution of organs derived from epithelial tissues.

3.2 The Loci of Phenotypic Evolution

In the last decades, great advances were made in identifying the genes and mutations that are underlying evolutionary relevant phenotypic variation between species or populations (reviewed and listed in Martin & Orgogozo, 2013). This progress relied on candidate gene approaches or unbiased genetic/genomic mapping approaches (eg, association and QTL (quantitative trait locus) mapping studies). How do these studies inform us about the genetic basis of the evolution of organs derived from epithelial tissues?

3.2.1 Changes in the Number of Organs

Vertebrate skin appendages are typically repeated in different parts of the body (Chuong et al., 2006) and can produce mineralized or keratinized structures, eg, scales, hair, feathers, and teeth. Changes in the number of these ectodermal appendages are frequent in vertebrate evolution, and the underlying genes have been identified in a few cases. It is remarkable that the five cases illustrated later all involved genes that are directly related to signaling pathways known to regulate the number and spacing of ectodermal placodes (Bmp, Fgf, Eda).

A loss of function mutation in the transcription factor Foxi3—a target of the Ectodysplasin A (Eda) pathway (Shirokova et al., 2013)—caused severe loss of hair and teeth in three dog breeds (Drogemuller et al., 2008); the loss of function of a receptor of Fgf (fgfr1a) correlates with complete loss of scales in two independently domesticated carp lineages (Rohner et al., 2009), while variation of this gene may be involved in the natural scale loss in Phoxinellus (Daane, Rohner, Konstantinidis, Djuranovic, & Harris, 2016). In contrast to these complete loss of functions, a cis-regulatory change increasing Bmp6 expression is associated with a twofold increase in the number of teeth in a benthic freshwater stickleback population (Cleves et al., 2014); and cis-regulatory variation in the ligand Eda and its receptor Edar is associated with severe reduction of armor plates in stickleback freshwater populations (Colosimo et al., 2005) and prickling in sculpin species (Cheng, Sedlazek, Altmuller, & Nolte, 2015). A mutation in Bmp12/ Gdf7 is associated with loss of neck feathering in some chicken (Mou et al., 2011). This gene displays markedly elevated expression in the embryonic skin due to a cis-regulatory effect of the causative mutation. The specific loss of feathers on the neck is surprising since Bmps presumably act as inhibitors in the activation–inhibition mechanisms regulating the size and spacing of feather follicles in the embryonic skin. However, Mou et al. (2011) show that a selective production of retinoic acid (RA) by neck skin potentiates Bmp signaling, making neck skin more sensitive than body skin to feather inhibition. The distinction in RA expression between neck skin and the rest of the body is cryptic because its effect on feathering is not revealed until Bmp levels are homogeneously increased. This cryptic variation in RA expression may have facilitated the evolution of bare necks, since even a mutation changing homogeneously the activator–inhibitor balance will easily have a heterogeneous spatial effect: affecting neck feathering while preserving body feathering. Interestingly, bare necks evolved many times independently, notably in tropical bird species.

3.2.2 Changes in Size and Shape of Organs

Generally speaking, phenotypic variation in shape (or size) tends to be less studied as compared to less integrated traits (eg, pigmentation intensity and patterns). One-third of the mutations listed by Martin and Orgogozo concern morphological evolution (384 of 1008, including 281 in metazoans). Yet, we counted only 27 mutations (for a total of 18 genes) involved in body or organ shape changes and 25 mutations (for a total of 23 genes) involved in body or organ size changes. Only four case studies have pointed genes associated with changes in size and shape in epithelial tissues directly: three of them deal with insect wings.

In D. melanogaster populations, about 10% of the natural variation in wing size along latitudinal clines is due to polymorphism in the Drosophila cold acclimation gene (Dca)/regucalcin gene (Lee et al., 2011; McKechnie et al., 2010). The gene functions in intracellular calcium homeostasis, and, interestingly, it seems to act as a tumor suppressor in many epithelial tissues (Yamaguchi, 2015). Regarding shape, QTL mapping of the intraand interspecific variation in Drosophila wing shape has led to the identification of many QTLs (Matta & Bitner-Mathe, 2010; Mezey, Houle, & Nuzhdin, 2005; Palsson & Gibson, 2000; Weber et al., 2001, 1999; Zimmerman, Palsson, & Gibson, 2000), but the genes underlying these many QTLs were not identified. There is also little overlap between studies, a typical situation when mapping genetic variation associated with shape variation. In a microarray study, Weber et al. (2008) studied multiple

replicates of one population. They selected lines with opposite changes in wing shape in the lab (divergent selection) and replicated their artificial selection five times with flies from Massachusetts, and one time with flies from California. Then, they performed pairwise comparison of gene expression in wing imaginal discs for each pair of lines to identify candidate genes for shape variation. They found virtually no overlap between all six replicates and therefore argue that most expression differences with statistically important effects on wing shape are different in the two populations. However, studies further focusing on the Egfr locus show that a putative regulatory polymorphism is associated in wild populations with continuous variation in the shape of a central intervenal region of the wing (Dworkin, Palsson, & Gibson, 2005). This is consistent with a late role of the Egfr pathway in regulating vein/intervein formation (Crozatier, Glise, & Vincent, 2004). In addition, QTL mapping of male-specific variation in wing shape and size between two species of Nasonia (wasps) identified cis-regulatory changes in Upd-like, a homologue of the Drosophila Unpaired gene (Upd) (Loehlin & Werren, 2012). In D. melanogaster, Upd was originally implicated in mutants that displayed small eyes and abnormal wing development phenotypes. These phenotypes were interpreted as perturbations of cell proliferation. Similarly, Loehlin and Werren (2012) suggest that Upd-like affects wing size and wing allometry via the regulation of proliferation (overall and differential growth rates). However, further work has showed that the role of Upd is more complex. In D. melanogaster, Upd binds both to the ECM and to a membrane-bound receptor, is regulated by ECM components, and is known to be capable of activating the Jak/Stat signaling pathway (Harrison, McCoon, Binari, Gilman, & Perrimon, 1998; Hayashi et al., 2012; Zhang, You, Ren, & Lin, 2013). This pathway has versatile roles in morphogenesis, inflammation, and epithelial tissue homeotasis in both vertebrates and invertebrates (Amoyel, Anderson, & Bach, 2014; Hombria & Sotillos, 2013; Hou, Zheng, Chen, & Perrimon, 2002). The cellular role of the Jak/Stat signaling pathway is poorly understood, as it seems to promote different target genes and different cell behaviors (cellular rearrangements, cell shape changes, or cell migration), depending on the context (Hombria & Sotillos, 2013). It also seems to modulate invagination and tissue folding. As such, the Jak/Stat pathway is similar to the other signaling pathways discussed earlier: it is a nonlinear regulatory gene network, it is context dependent, and its secreted ligands are regulated by the ECM, probably in an integrative way.

It is noteworthy that the three genes associated with change in insect wing shape/size play a role in epithelial tissue homeostasis and cancer. Both the Jak/Stat pathway and the Egfr pathway have been reported to interact with the mechanosensitive Hippo pathway (Karpowicz, Perez, & Perrimon, 2010; Ren et al., 2010; Sarikaya & Extavour, 2015; Shaw et al., 2010). The fourth case for which a change in organ size/shape was reported is particularly interesting with regard to the role of the ECM: Has2, an enzyme synthesizing one of the key components of the ECM— hyaluronan—is associated with skin folding and thickening in shar-pei¨ dogs and mole rats (Akey et al., 2010; Faulkes, Davies, Rossiter, & Bennett, 2015; Tian et al., 2013). Moreover, Has2 deregulation is associated with a poor prognostic of breast cancers, as it contributes to metastasis (Heldin, Basu, Kozlova, & Porsch, 2014), and it plays also a role in cell migration during zebrafish gastrulation (Bakkers et al., 2004).

3.2.3 Indirect Evidence for Changes in the Shape of Organs

Changes in the branching of internal organs (eg, lung, kidney) could, in principle, have an adaptive, “physiological” role. Although several genes are known to influence the number and length of branches in various diseases and mutants, no gene associated with such change has been reported so far in an evolutionary context, possibly because associated traits cannot be quantified easily.

In contrast, changes in skin and skin derivatives, as discussed earlier in the case of teeth, are more tractable and they were the subjects of several mapping studies. Variations in hair, feather, or tooth morphology, but also variation in milk production, could have two origins: (1) variation in terminal production of the trait, eg, production of keratin, enamel, or milk by specialized cells; (2) variation in epithelial morphogenesis, eg, size and shape of, respectively, hair/feather follicle, enamel organ, or mammary gland. Hairs provide a well-documented example. Most of the known mutations responsible for evolution of hair morphology are repeatedly found in the same genes: they act on terminal hair production (typically, mutations in keratins, Cadieu et al., 2009; Gandolfi et al., 2010; Ng et al., 2012) or hair follicle cycling physiology (typically, mutations in FGF5 associated with long hair, via an increase of the hair growth phase; Drogemuller, Rufenacht, Wichert, & Leeb, 2007). However, a subset of mutations may act by changing hair follicle shape. This is likely the case for the adaptive variant of EDAR found in Asia and affecting hair thickness (Fujimoto, Kimura, et al., 2008; Fujimoto, Ohashi, et al., 2008). This variant has

increased signaling capacities, and transgenic mice with elevated EDAR signaling show a hair phenotype resembling the one observed in humans and resulting from an enlarged follicle (Mou et al., 2008). This could be the case as well for the FGFR2 variant in humans (Fujimoto et al., 2009) and the Rspo2 variant in dogs (Cadieu et al., 2009). It remains to be demonstrated how hair follicle shape may be modified in these variants, but the three targeted pathways (EDA, FGF, and WNT, respectively) are pathways typically associated with the morphodynamic development of the hair follicle. Similarly, variation in milk production in bovins and ovins was related to variation in transporters and enzyme used for milk production (Cohen-Zinder et al., 2005; Garcia-Fernandez et al., 2011; Grisart et al., 2002), but also variation in the coding sequence of the prolactin receptor and the growth hormone receptor (Viitala et al., 2006). Those two receptors are known to stimulate ductal outgrowth in the mammary gland, yet it is not known whether the increased milk production is due to increased branching.

In conclusion, the genetic changes at the origin of the morphological evolution of organs derived from epithelial tissues have been identified only in very rare occasions. Furthermore, the corresponding cases are confined to domestication and human evolution, or regressive evolution (ie, organ loss), which are not necessarily representative of evolution in general. Most cases involve cis-regulatory changes in a few signaling pathways that may appear as hot spots (eg, Eda, Fgf, Bmp). Although this may reflect a sampling bias toward developmentally well-known pathways, the modularity of cisregulatory changes may facilitate evolution by counteracting the pleiotropy of developmental genes (Carroll, 2008; Stern, 2000). Interestingly, the very few genes that were specifically linked to size and shape changes all appear to be associated with epithelial cancers (ie, Dca, Upd-like, Has2). This may suggest—if proof is needed—that the morphodynamic mechanisms presented earlier are fully relevant for the evolution of organs derived from epithelial tissues. Yet, in order to draw firm conclusions we will need to identify not only the genes involved in other cases but also the cell behaviors they mediate.

Despite the relevance of these findings for the study of morphological evolution, the scarcity of examples makes it difficult to draw specific rules for the genetics of organ evolution. The example of Drosophila wing shows how quantitative genetics approaches are limited by the intrinsic complexity of the genetics underlying shape differences. This suggests that the evolution of these organs, most notably their variational properties and their integration (correlation among traits), will be understood if and only if we study their morphogenetic mechanisms.

4. The evo-devo perspective on the diversification of organs derived from epithelial tissues

4.1 Development Structures Phenotypic Variation and Evolution

Evolutionary tinkering and the integrative nature of development seem to ensure that mutations at any hierarchical level can result in a fully developing, reproducing, living form. For example, mutations that lead to extra fingers usually lead to at least partly functioning digits with the appropriate muscles, nerves, or blood vessels (Lieberman & Hall, 2007). This may have provided evolutionary shortcuts in the production of novel morphologies (Valentine, Jablonski, & Erwin, 1999). The self-organizing nature of development does not only have the capacity of rescuing otherwise presumably hopeless monsters into integrated, functioning phenotypes, but it also structures and limits the range of possible phenotypic variation. As a consequence, some morphologies are more readily generated than others. The corresponding developmental “constraints” or “biases” have been the subject of many debates (Alberch, 1980, 1989; Alberch & Gale, 1985; Amundson, 1994; Beldade, Koops, & Brakefield, 2002; Goodwin, 1988; Gould & Lewontin, 1979; Kauffman, 1993; Oster et al., 1988; Urdy, Wilson, Haug, & Sánchez-Villagra, 2013; Webster & Goodwin, 1982), but empirical and theoretical studies remain sparse. The debate about the relative role of natural selection and developmental biases in ordering phenotypic variation and driving phenotypic evolution relies on two fundamentally different assumptions about the relationship between genotype and phenotype—whether linear or nonlinear, respectively—and the kind of morphological variation that it may produce: a linear genotype/ phenotype mapping may result in gradual, unbounded phenotypic variation, whereas a nonlinear mapping implies discrete and limited variation (Salazar-Ciudad, 2006a, 2006b).

Taxonomists and evolutionary biologists make every day more or less explicit assumptions on the relative ease of evolving a particular shape from a putative ancestral morphology. The building of phylogenetic trees— relying on morphological or molecular traits—is always based on some measure of “evolutionary distance.” If the considered characters are independent, parsimony leads to define

“evolutionary distance” as some sort of minimal count of character differences. Yet, in highly integrated organs such as mollusc shells and vertebrate teeth, upon which most of our understanding of fossil microevolution is based, morphological characters are not always independent but may covary. Recognizing patterns of covariation and modularity is the first step toward the construction of trees that will better reflect the true evolutionary distances between taxa, that is, taking into account not only the morphological differences but also the developmental differences needed to achieve these morphological differences.

We argue here that a better characterization of development, understood as the complex, integrative mapping between the genotype and the phenotype, is necessary if one wants to fully understand the evolutionary consequences of the recent spectacular advances of molecular biology. Evolutionary developmental biology’s earliest discoveries were about large-scale modifications in body plan, as the development of phylogenetically distant organisms was compared first. As the working resolution improves, the development of more closely related organisms is now scrutinized and researchers are increasingly exploring the developmental genetic bases for the variation within populations or across closely related species (Brakefield, 2003; Frankino, Zwaan, Stern, & Brakefield, 2005; Lieberman & Hall, 2007; Shapiro et al., 2004; Stern, 1998, 2000). This recent change of research emphasis from the sorting of phenotypic variation by natural selection to the production of that variation through development (Beldade et al., 2002) is most welcomed. In our opinion, the wealth of empirical and experimental studies would now also benefit from sustained efforts toward modeling, as most of the studied processes involve too many components and interactions for us to intuitively understand. The following example has been selected to highlight the added gain in understanding that such theoretical works can bring about.

Possibly because they mediate interactions with the environment, vertebrate ectodermal appendages such as teeth, fish scales, hairs, feathers, or mammary glands evolve rapidly and exhibit a high diversity. Hence they are particularly well suited for evolutionary studies, especially if they mineralize and consequently fossilize readily, as it is the case for teeth and fish scales (Donoghue, 2002). Not only the fossil record of teeth is good and abundant but their development is also considered the simplest of all vertebrate organs (Stock, Weiss, & Zhao, 1997), and it is now relatively well understood (Jernvall & Thesleff, 2012 and references below). Moreover, many aspects of tooth development have been shown to be common to the development of other ectodermal appendages (Thesleff et al., 1995; Chuong et al., 2006), which means that breakthroughs in the understanding of teeth’s evolution and development may be readily exported to other organs, making them one of the best model systems for evolutionary developmental studies.

Teeth are the most readily preserved parts of vertebrates in the fossil record and as such represent most of what we know from extinct mammals. Hence it is of no surprise that phylogenetic analyses of extinct mammals rely heavily on characters based on dental features (Asher et al., 2005; Luo, Cifelli, & Kielan-Jaworowska, 2001; Meng & Wyss, 2001; O’Leary et al., 2013). As mentioned earlier, earlier studies (Kangas, Evans, Thesleff, & Jernvall, 2004; Luo, 2007; O’Keefe & Wagner, 2001; Wake, 1989) have demonstrated that tooth and tooth row development can be highly integrated, which results in covariation patterns between some, if not all, of these dental characters. Yet, in phylogenetic analyses, characters are typically considered independent (Doyle, 1997; Felsenstein, 1973; Kluge & Farris, 1969; O’Keefe & Wagner, 2001), which may largely bias interpretations of phylogenetic interrelationships. In order to weigh the influence of particular character dependencies on the distribution of phenotypic variation and consequently on evolutionary hypotheses, in vivo or in silico experiments must be conducted to evaluate the morphological effects of slight changes in development and/or molecular signaling. For instance, Salazar-Ciudad and Jernvall (2010) suggest that despite the complexity of development, changes in one signaling parameter could underlie the variation observed among individuals of ringed seals and changes in one tissue growth parameter could underlie the variation among tooth series in their jaws. The produced 3D morphologies and 3D patterns of gene expression can be monitored along ontogeny and compared with experiments. This enables also the reexamination of fossils in a developmental perspective (Urdy et al., 2013). Experiments of this kind are also useful to characterize the linear or nonlinear nature of the genotype/phenotype mapping, and hence the topology of the space of achievable morphologies. To date, only a handful of such studies can be reported (Kavanagh, Evans, & Jernvall, 2007; Kavanagh et al., 2013; see Urdy et al., 2013 for review). We focus below on a recent work by Harjunmaa et al. (2014), which we think is exemplary of how experiments on explants and theoretical models can be successfully integrated to explore the developmental bases of hypothesized evolutionary transitions and to produce insightful predictions about the probability of particular character-state modifications.

4.1.1 In Vitro and In Silico Recapitulation of Phenotypic Evolution as an Experimental Validation of Trait Covariation

Harjunmaa et al. (2014) used mice as a model organism and in particular mice carrying a spontaneously occurring null mutation in Eda. This gene modulates the morphogenesis of most ectodermal organs, including teeth, hairs, feathers, and mammary glands. Eda is also implicated in the fine-tuning of the morphogenesis of fish scales, a mineralized organ with a good fossil record (Harris et al., 2008). Hence, Eda had already been suggested to play a role in the evolution of fishes (Schmid & Sánchez-Villagra, 2010). Eda had been previously demonstrated to have also relatively subtle effects on tooth morphology: Eda deficiency in mice leads to smaller, less numerous teeth with missing cusps, while its overexpression in ectoderm via the K14 promoter leads to larger and supernumerary teeth (Charles, Pantalacci, et al., 2009; Grueneberg, 1965; Kangas et al., 2004; Laurikkala et al., 2001; Mikkola & Thesleff, 2003; Mustonen et al., 2003; Peterkova, Lesot, Viriot, & Peterka, 2005; Pispa et al., 1999; Tucker, Headon, Courtney, Overbeek, & Sharpe, 2004). Historically, the Eda pathway was indeed one of the first signaling pathways that could be used to induce tooth morphological modifications without complete loss of teeth. Eda has also pleiotropic effects, which means the induced modifications are informative about the potential character dependencies that are to be characterized. Harjunmaa et al. (2014) gradually added Eda in culture to tooth explants of their null Eda mutant mice. The wild-type “morphology” (as reduced to the number of cusps) is restored if sufficient Eda is added. Increasing dosage accelerates the initiation of some cusps by accelerating the differentiation of ectodermal cells into nonproliferative enamel knots cells, thus creating secondary enamel knot signaling centers. Increased dosage also leads to a larger primary enamel knot and subsequently to more cusps. In order to analyze the induced morphological modifications, these authors used character states comparable to the ones used in phylogenies, in particular the presence/ absence of particular cusps and the height of the talonid, a characteristic feature of tribosphenic molars. In tribosphenic teeth, the talonid is found posterior of the trigonid and bears three cusps. Before the evolution of the talonid, a single cusp occupied that position. A similar, single-cusped condition is recapitulated in Harjunmaa et al.’s cultured teeth at low Eda dosage. Harjunmaa et al. (2014) show also that talonid height and cusp number covary developmentally, which they also confirm by morphometric analyses on 35 extant murine rodents and 32 extant carnivorans. Whereas large variation of the talonid structure and size can be reproduced by small changes of Eda signaling, the geometry of the trigonid appears immune to these changes. This has major implications for the evolution of the mammalian tribosphenic teeth that are diagnosed by their derived talonid features (Harjunmaa et al., 2014). Not only the transitions observed in the fossil record can be reproduced experimentally but all these transitions could theoretically be induced by different dosages of the same signal (Eda). Furthermore, Harjunmaa and colleagues perform in silico computer simulations using the model of Salazar-Ciudad and Jernvall (2010). Remarkably, the simulations reproduce the observations, including the full range of transitions observed both in the fossil record and in the experiments.

4.1.2 Numerical Cell-Based Models as a Tool to Predict Evolutionary Changes

The complex nature of the model of Salazar-Ciudad and Jernvall (2010) renders the outcome unpredictable analytically: one needs to run the simulation to know the result. Yet, some behaviors of the model can be predicted to some extent. For instance, the distance between two cusps (or equivalently, two enamel knots) is constrained by the diffusion parameters. Everything else being kept constant, an increase of the domain size will almost necessarily lead to the formation of more cusps. It is striking in Harjunmaa et al. (2014) that the primary enamel knot size at culture day 2 predicts the number of cusps at day 7. This suggests that the network controlling cusp initiation is sensitive to domain size: a larger primary enamel knot at day 2 leads to a larger tooth at day 7, which, as expected in a reaction– diffusion-like system, translates into more numerous cusps. This would also imply that the tooth is larger at intermediate times, which may explain easily the observed heterochrony of cusp initiations. Mice deficient for either Eda or Edar (its receptor) display no apparent abnormalities in the formation of the dental placodes (Ahn, 2015; Kangas et al., 2004; Pispa et al., 1999), so the Eda pathway does not seem to be involved in the induction of placodes. Both Eda/NF-kB and Wnt/β-catenin signaling pathways (the latter being upstream of the Eda pathway; reviewed in Sadier, Viriot, Pantalacci, & Laudet, 2014), when stimulated, increase cell motility and the number of cells committed to placodal fate. Moreover, Ahtiainen et al. (2014) have studied the processes by which hair placodes form and concluded that not proliferation but cell compaction and

centripetal migration were involved in the recruitment of placodal cells. So, the Eda pathway seems to regulate domain size by controlling the number of cells migrating into a placode.

Systems, like this one, that can be modeled by a reaction–diffusion-like mechanism have generic (common) properties. In particular they are sensitive to domain size and shape in a way that is partly predictable. This allows us to explore efficiently how development structures phenotypic evolution if, as here, in vivo experiments can be combined with a relevant cell-based model. We expect that organs other than teeth will become amenable to such approaches in the near future. This will allow us to predict the likely direction of future evolutionary changes and to critically reassess the fossil record.

4.2 From Developmental Models to Evolutionary Genetics

Historically, evolutionary genetics has been largely dominated by quantitative genetics and population genetics, which tend to focus on additive variation. Theoretical approaches built to deal with complex, nonadditive systems emerged more recently (Rice, 2008). They put a stronger emphasis on the reciprocal influence of development and genetic architecture on evolutionary paths. The very nature of morphogenetic mechanisms, as captured by the models discussed earlier, may predict the genetic architecture of the corresponding phenotypic traits, the role of epistatic interactions, or the likelihood of cryptic genetic evolution. How may the genetic evolution of traits based on a morphodynamic mode of development differ from that of others relying on more linear modes of development? Shall we expect qualitative or quantitative differences?

4.2.1 Current Models of Morphogenesis Lack a Gene to Parameter Mapping

An important limitation of Salazar-Ciudad and Jernvall's model (2002, 2010) and similar models is that they incorporate only a very simplified version of the involved genetic networks. For instance, each of SalazarCiudad and Jernvall's model parameter encapsulates the actual effects of many genes. Only one pair of activator/inhibitor is coded, although we know that multiple pathways are involved, with multiple secreted ligands interacting with multiple antagonists in a context where multiple ECM molecules may impact their diffusion/degradation, a complexity that may add redundancy and robustness to the system (Felix & Barkoulas, 2015). The current reduction is sufficient (and certainly preferable) for the in silico exploration of the developmental structuration of phenotypic variation. But it precludes hitherto access to one of the central questions of evolutionary biology: the relationship between genetic and phenotypic variations (see Felix, 2012). One would like to have bona fide gene-to-phenotype models incorporating morphogenetic mechanisms to make predictions about how morphodynamic development may shape the genetic architecture of the corresponding traits during evolution (in terms of nature and extent of epistatic interactions, cryptic genetic evolution and drift) and to make predictions about how they combine to influence adaptive and neutral phenotypic evolution. A first step might be adding extra layers to existing models, for example, by introducing a gene-to-parameter relationship only for some parameters (eg, the genetic kernel of activation–inhibition in the tooth model).

In the meantime, we can deduce from these models that organs derived from epithelial tissues may display a number of generic properties. Central to our reasoning is the nonlinear behavior of such morphodynamic systems, which implies that very small changes in parameters may have large impact on the phenotype, while larger changes may have no impact (ie, Salazar-Ciudad, 2006a, 2006b; Salazar-Ciudad & Jernvall, 2004, 2010). In other words, such systems can be quite robust to large parameter changes but at the same time be very sensitive to a small parameter change. This has many consequences that we propose to explore here.

4.2.2 From Morphodynamic Systems to Genetic Drift and Cryptic Evolution

We have seen that complex shape and evolutionary relevant shape variation of organs derived from epithelial tissues can be reproduced with morphodynamic models, built on a few generic rules and a few parameters. It suggests that the genetic complexity underlying shape and shape variation reflects a multiscale problem (integration from molecules to tissue behavior), where all levels matter. As a consequence, whether genetic variation has fitness effects or not is determined at the system level, not at the level of individual genes.

Morphodynamic systems are not only robust to certain parameter changes but also robust to changes in the ratios of parameters. In general, ratios of morphogenetically relevant parameters are likely to be more

important than absolute values. Put together, this suggests that such systems are especially prone to genetic drift. Concretely, the genes involved, their expression levels, their isoforms, etc., may drift quite extensively: as far as the overall balance is maintained, the phenotype will remain unchanged. On a microevolutionary scale, this drift will favor in the accumulation of cryptic genetic variation (or conditional-effect variation; Paaby & Rockman, 2014) among and between populations. On a macroevolutionary scale, this genetic drift will favor developmental systems drift or cryptic developmental evolution (True & Haag, 2001).

Furthermore, in a recent evolutionary model of cancer, Rozhok and DeGregori (2015) recall that the drift–selection balance is dependent on population size. The smaller the effective population size, the larger the change in phenotype need to be selected upon. Thus, in large populations, minute phenotypic changes produced by mutations can be acted on by selection, while in small populations, most mutations are expected to be neutral to selection, at least if their phenotypic effect is relatively small. Interestingly, these authors recall that the compartmental organization of stem cells is strikingly different in epithelia and in other tissues: in epithelia, pools of stem cells are small and fragmented (less than 20 cells), while the pools of hematopoietic and mesenchymal stem cells are large and well mixed in the bone marrow (with estimates ranging from ten thousands to hundreds of thousands of cells). As a consequence, the drift–selection balance is expected to be higher in epithelial tissues than in other tissues (Rozhok & DeGregori, 2015). In our view, the combination of small stem cell populations and morphodynamic development is then all the more likely to lead to genetic drift and to the accumulation of cryptic genetic variation, possibly well before the onset of carcinoma formation.

4.2.3 From Morphodynamic Systems to Context Dependence of Mutations and Evolutionary Novelty

Another likely consequence of morphodynamic systems is that the effect of mutations may be strongly context dependent. Importantly, this dependence may not be limited to epistatic effects between two (or a limited number) of loci, but extended to many loci not to mention the context-dependence induced by the environment. Cryptic genetic variation may be released upon change in environment or genetic background (Bergman & Siegal, 2003; Hermisson & Wagner, 2004; McGuigan, Nishimura, Currey, Hurwit, & Cresko, 2011; Rutherford & Lindquist, 1998; Waddington, 1956). In our case, a mutation may be revealed or silenced depending on the geometry of the tissue in which the developmental networks are operating. It means that depending on the differences in accumulated cryptic genetic variation in two populations, a newly introduced mutation may be neutral to selection in one population, while the same mutation may be selected upon in the other population. This would result in an apparent saltatory evolution and would put a strong focus on the newly appeared mutation, whereas in fact more genetic elements and more gradual evolutionary processes are involved in the abrupt phenotypic change.

4.2.4 From Morphodynamic Systems to Adaptive Evolution

Morphodynamic models offer the possibility to perform in silico exploratory experiments of the kind that would be totally impossible in the lab. For instance, Salazar-Ciudad and Marin-Riera (2013) simulated the evolution of phenotypic variation in molar shape in different populations under various types of natural selection criteria. They demonstrate that, because of the complex nature of the mapping between the genotype and the phenotype in such morphodynamic simulations, natural selection could not lead to adaptive morphologies in which many of the dental traits are adaptive. In other words, “optimal phenotypes” that require a unique combination of many trait values are unlikely to be achieved by natural selection, unless the initial phenotypes in the population are already very close to the “optimal phenotype.” Natural selection would lead to “optimal phenotypes” solely by operating on a few traits or a single global shape descriptor. This means that most tooth traits taken individually might be nonadaptive. This means also that the shortest evolutionary trajectory between an initial phenotype and an “optimal” morphology (presumably an adaptation to a specific diet) can be quite counterintuitive.

Nevertheless, if the phenotype is close enough to the “optimal phenotype” and if population size is large enough, natural selection could operate on more discrete traits. This suggests that it is theoretically possible that different selection criteria acting on different levels of detail are involved at different times during evolution. The addition of a gene-parameter mapping in such models may inform us on the type of genetic changes that are likely to occur at different phases if such an optimization process is assumed. Are mutations associated with major transitions more likely to be large effect mutations in key developmental genes such as those discussed earlier? Are the genetic changes associated to fine-tuning and optimization

rather linked to the many genes that interact with them? Clearly it is too early to answer these questions
Additionally, the same model (Salazar-Ciudad & Jernvall, 2010) was also used to identify another potential way the development may structure the evolution of phenotypic variation: Harjunmaa et al. (2012) show that everything else being equal, an increase in dental complexity is harder to achieve than a decrease in complexity and hence may necessitate more mutations.

4.2.5 Pleiotropy and the Adaptive/Neutral Phenotypic Evolution of Organs

Although ectodermal organs are very diversified within a body, their development relies on the same pathways. Pleiotropy is thus a major issue for their evolution. Traditionally, pleiotropy is considered as a brake to adaptative evolution, since genetic mutations that would be advantageous for a trait are likely to be deleterious for other traits, leading to nonselection of the corresponding traits and ultimately to a trade-off (Carroll, 2008). Therefore, for pleiotropic genes, it is predicted that only nonpleiotropic mutations should be evolutionary relevant. Cis-regulatory sequences, that introduce modularity into pleiotropic genes, are thus considered to play a more important role than coding sequences in morphological evolution (Stern, 2000). It is generally overlooked, however, that pleiotropy could instead favor adaptive evolution in some cases. A derived EDAR allele has been positively selected and almost fixed in Asian and Native American populations (Bryk et al., 2008; Fujimoto, Kimura, et al., 2008; Kamberov et al., 2013). It has been associated with a phenotype with thick hair, shovel-shaped incisors and more numerous sweat glands (eccrine glands) and possibly increased mammary gland branching. In this example, several traits could be advantageous traits (at least eccrine gland density and mammary gland branching), and others may simply follow without major deleterious consequences (eg, tooth morphology). Therefore Kamberov et al. (2013) interestingly propose that selective forces related to different traits may have acted on this variant either simultaneously or successively during its long history. This suggests that mutations with pleiotropic effect could, in contrast to the common view, help fixation of derived alleles and thus favor in the meantime adaptive and neutral morphological evolution in different body parts. For instance, Rodrigues et al. (2013) propose that modifications of the Eda pathway could underlie the evolutionary origin of stephanodonty (aka presence of crests between the molar cusps) in lineages of murine rodents. Such dental changes are classically thought to reflect an adaptation (here, to herbivory). Alternatively, the fur, not the associated dental traits, may have been positively selected: indeed one could argue that a change toward thicker fur might have helped these rodents to cope with reconstructed colder climates. As suggested by Kamberov et al. (2013), selection may have acted on both traits in combination. Thus, we should consider possible synergistic effects when interpreting putative adaptations.

The properties of morphodynamic systems suggest another exception to the common view on pleiotropy. It is generally assumed that a mutation in a pleiotropic gene that has large effects in a particular organ will have large effects in many other organs as well. However, because the nonlinear and geometric effects are expected to be largely organ specific, it is instead expected that such mutation will have qualitatively different effects in different organs within the body. Pleiotropic molecular effects do not equal pleiotropic phenotypic effects (see above the Bmp12 mutation in body vs neck feathering). How frequent is this phenomenon? Cis-regulatory mutations with organ-specific effects provide an opportunity to assess the frequency at which this phenomenon occurs: How many of them actually drive organ-specific expression changes? How many drive more pleiotropic expression change, yet organ-specific phenotypic change? We suggest that this should be examined systematically.

Finally, Pavlicev and Wagner (2012) proposed the “selection– pleiotropy–compensation” model for pleiotropic genes. According to this view, adaptation does involve not only the fixation of a given mutation associated with an advantageous trait but also the elimination of many other alleles to limit the deleterious effects of the said mutation on other traits (eg, other organs). This model might be highly relevant for skin appendages, because pleiotropy is expected and compensatory evolution might be easy if, as we predict, there is vast amount of standing cryptic genetic variation. Altogether, these effects may have facilitated the independent diversification of ectodermal appendages, although they share largely the same genetic toolkit.

4.2.6 Pleiotropy Can Shed Light on the Function of Genes

Studying in details the basis for pleiotropic effects in a pathway is likely to shed light on its functional role in terms of induced cellular behavior. For instance, since Eda/NF-kB are involved in the morphogenesis of several organs, comparative analyses of these organs’ development may be highly informative. We have seen earlier that the Eda pathway seems to regulate the number of cells that migrate to

form the hair placodes, and by analogy, possibly all placodes of skin appendages. NF-kB is a highly pleiotropic pathway, found downstream of Eda, as well as downstream of tumor necrosis factor (Tnf) immune receptors (homologous to the Eda receptor). The NF-kB pathway is primarily known for its major role in inflammation and immunity (Gilmore & Wolenski, 2012): it regulates the expression of chemokines that stimulate the migration of leukocytes to local inflammatory sites (Billottet, Quemener, & Bikfalvi, 2013; Lefebvre & Mikkola, 2014). Interestingly, several chemokines (cxcl10, cxcl11, and possibly cxcR3) were shown to be expressed in hair placodes and to be upregulated by Eda/NF-kB (Lefebvre, Fliniaux, Schneider, & Mikkola, 2012). Several studies suggested also that these chemokines regulate keratinocyte migration and wound healing (Kroeze et al., 2012; Yates et al., 2009) and drive epithelial cell migration and invasion into the mesenchyme in breast cancer (Ma et al., 2009; Shin, Nam, Lim, & Lee, 2010). Put together, these data suggest that, evolutionary speaking, the Eda pathway could be considered a Tnf member module that induces inflammatory-like responses in locally induced thickenings of the skin (placodes) via the production of chemokines that act as local chemoattractors orienting and accelerating migration of neighboring cells. Actually, the ability of the Eda pathway to control morphogenetic cell behavior likely reflects the conservation of an ancient cytoskeletal-control machinery that was coopted by the other Tnfs to regulate part of the apoptotic and inflammatory response in multicellular animals (Mathew, Haubert, Kroenke, & Leptin, 2009). Since Eda is also involved in the morphogenesis of the mammary gland (Lindfors, Voutilainen, & Mikkola, 2013; Voutilainen et al., 2015) and in the initiation of breast cancer in mammals (Nam et al., 2013), the full characterization of its function via such comparative analyses may shed new light in breast cancer research.

4.2.7 The Likelihood of Phenotypic Convergences in Evolution

On the one hand, the phenotypic variation in integrated systems is not expected to be isotropic but biased in preferred developmental directions. At least in the case of the mammalian molar, patterns of variation also seem to be shared by distant species (Harjunmaa et al., 2014), suggesting that contrary to the underlying genetics, the developmental system itself could be conserved (ie, the model would hold true) over long evolutionary times. On the other hand, in these systems, different genes may produce similar parameter changes and even different parameter configurations may produce a similar phenotypic output. Although Harjunmaa et al. (2014) demonstrate convincingly that relatively small changes in Eda signaling can have large correlated effects on the morphology of mice molars and can recapitulate ancient evolutionary informative morphological transitions, no genetic data are available for such old fossils, and hence, there is still no direct evidence that the Eda pathway was indeed involved in those evolutionary transitions. Other pathways are also known to be able to recapitulate evolutionary transitions: for instance, RA (Gibert et al., 2015) and Fgf3 (Charles, Lazzari, et al., 2009). More generally, several studies have pointed the resemblance of phenotypic changes observed between wild type and mutants with those observed in evolutionary transitions (forward or backward; Charles, Lazzari, et al., 2009; Charles, Pantalacci, et al., 2009; Harjunmaa et al., 2014; Gibert et al., 2015; Kangas et al., 2004; Marangoni et al., 2015; Rodrigues et al., 2013). Whereas these experiments provide invaluable information on patterns of covariation, reveal the developmental structure of the trait involved in the transition, and demonstrate that the fine-tuning of a single gene is sufficient to recapitulate evolutionary transitions, they do not imply that the gene or pathway was actually involved in the evolutionary transition that occurred: many other genetic modifications may result in the same effect and lead to evolutionary convergences.

Altogether, this suggests that morphodynamic, integrated systems are prone to homeomorphies, which are then expected to be the rule rather than the exception. This effect could be amplified by the genetic drift mentioned earlier.

5. Conclusion

Epithelial tissues are ideal to illustrate the interrelationships between development, evolution, and cancer formation. In particular, we have seen that development and evolution make use of relatively conserved signaling pathways. The same “morphogenetic routines” are being used and reused, often reiteratively in many different organs and organisms. We have seen also that development involves out-of-equilibrium processes, which underscores the importance of relative timing in orchestrating the morphogenesis of epithelial tissues and reflects in the relative preponderant use of heterochronies in explaining size and shape diversity in traditional evolutionary studies. We have seen also that most of the

mechanisms that are proposed to be involved in normal development and, by extension, in carcinogenesis are morphodynamic in nature, which implies they are best formalized and understood using computational models. In particular, cell-based models uniquely enable us to characterize the mapping between the molecular and the phenotypic scales. Such models have shown that morphodynamic mechanisms necessarily lead to discrete and biased phenotypic variation, which suggests that patterns of phenotypic integration should be studied in more details and should be compared with both predicted and observed evolutionary transitions. We note that such approaches are indeed rare, but we hope they can be extended to more systems in the near future. Owing to its central, integrative role in mediating mechanical regulation, we argue that the ECM should deserve more attention, in particular in such theoretical models, where its importance has been hitherto usually overlooked. Finally, we have suggested that morphodynamic systems may be prone to genetic drift, cryptic genetic variation, pleiotropy, and context-dependent mutational effects. These genetic properties, in conjunction with the generic aspects of morphodynamic systems, may explain why evolutionary convergences are the rule rather than the exception.

Acknowledgments
S.U. is funded by SystemsX.ch initiative, Transition Postdoc Fellowship “Morphogenesis of monolayer epithelia: models and experiments.” N.G. is funded by a French ANR @RAction grant (Project EvoDevOdonto). S.U. and N.G. contributed equally to this work. S.P. is funded by ANR grant (ANR 2011 JSV6 00501 “CONVERGDENT”) and CNRS. S.P. would like to thank Etienne Rajon for helpful discussions. We thank Virginie Orgogozo and Alexis Matamoro-Vidal for their constructive reviews.

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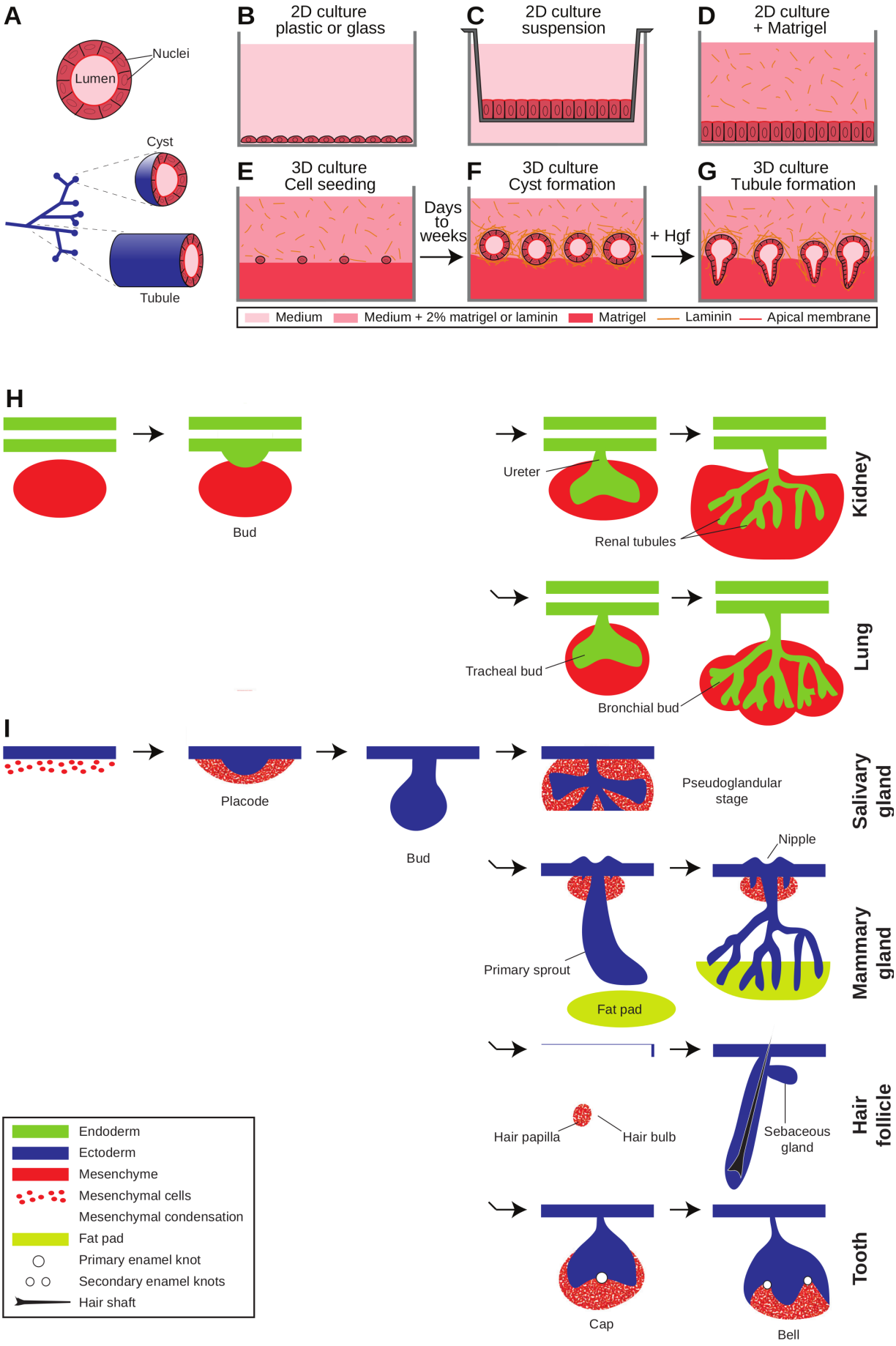
A
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E
3D culture
Cell seeding
Days
to
weeks
F
3D culture
Cyst formation
+ Hgf
G
3D culture
Tubule formation
Medium
Medium + 2% matrigel or laminin
Matrigel
Laminin
Apical membrane
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Bud
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Renal tubules
Kidney
Tracheal bud
Bronchial bud
Lung
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Placode
Bud
Pseudoglandular
stage
Salivary
gland
Nipple
Primary sprout
Fat pad
Mammary
gland
Hair papilla
Hair bulb
Sebaceous
gland
Hair
follicle
Cap
Bell
Tooth
Endoderm
Ectoderm
Mesenchyme
Mesenchymal cells
Mesenchymal condensation
Fat pad
Primary enamel knot
Secondary enamel knots
Hair shaft

Fig. 1 Morphogenetic routines involved in epithelial morphogenesis. (A) Internal organs of endodermal origin are made of two building blocks: tubules and cysts. A monolayer of polarized cells encloses a lumen. (B)–(D) 2D culture of epithelial cells. (B) Culture on plastic or glass. Cells adhere to the bottom of the well, are rounded, poorly polarize, and fail to differentiate. (C) Culture on porous membrane. Cells can polarize and differentiate as they can access nutrients through their basal side which adheres to the porous membrane. (D) With ECM components added to the culture medium, cells can polarize and differentiate. (E)–(G) 3D culture with Matrigel coated wells. (E) Seeding of individual cells on the Matrigel coating. (F) Cystogenesis, with lumen formation and polarization. (G) With Hgf added to the medium after cyst formation, tubules form and invade the matrigel. (H) In internal organs of endodermal origin such as lungs and kidneys, the epithelium bulges out of a tube (gut) and invaginates into the mesenchyme, where it starts branching reiteratively. (I) In ectodermal derivatives, the epithelium thickens and the mesenchymal cells condense, thus forming a placode, which invaginates into the condensed mesenchyme to form a bud. Then, reiterative branching occurs in glandular organs such as the salivary gland or the mammary gland, while in teeth morphogenesis the epithelium folds reiteratively. In hair follicle formation, the epithelium invaginates up to the point where it encloses the mesenchyme.

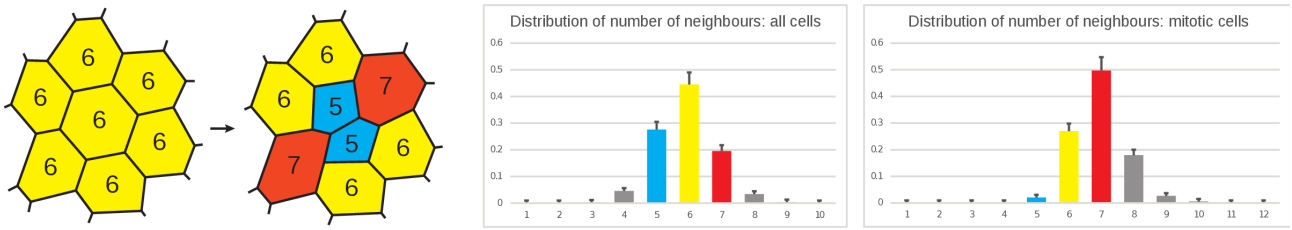

Fig. 2 Topological changes induced by proliferation result in conserved distribution of number of cell sides in metazoan monolayer epithelia. (A) Summary diagram of topology changes during cell division. Daughter cells can lose one side, while neighbors can gain one side. (B) Distribution of number of cell sides. (C) Distribution of number of cell sides of (pre)mitotic cells. (B) and (C) Experimental data from the Drosophila imaginal wing disc at larval stage 3. Errors bars assumes 10% miss-identification of number of neighbors. Reproduced from Aegerter-Wilmsen, T., Smith, A. C., Christen, A. J., Aegerter, C. M., Hafen, E., & Basler, K. (2010). Exploring the effects of mechanical feedback on epithelial topology. Development, 137, 499–506.