# Of Extracellular Matrix, Scaffolds, and Signaling: Tissue Architecture Regulates Development, Homeostasis, and Cancer

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## Key Words

tissue context, differentiation, mammary gland, microenvironment, morphogenesis, stromal-epithelial interactions

## Abstract

The microenvironment influences gene expression so that the behavior of a cell is largely determined by its interactions with the extracellular matrix, neighboring cells, and soluble local and systemic cues. We describe the essential roles of context and organ structure in directing mammary gland development and differentiated function and in determining the response to oncogenic insults, including mutations. We expand on the concept of "dynamic reciprocity" to present an integrated view of development, cancer, and aging and posit that genes are like the keys on a piano: Although they are essential, it is the context that makes the music.

## Contents



**Microenvironment:** local and systemic constituents surrounding a cell, including ECM, other cells, and soluble factors released locally or transmitted from other organs, such as hormones

## INTRODUCTION

The function of an organ relies upon the organ's constituent cell types and overall organization. It is the obvious uniqueness of this structure that distinguishes, e.g., a breast from a kidney and that directs the cells within the former to make milk and within the latter to filter blood and make urine; this is so despite the fact that they share an identical genome. But whereas tissue specificity is a certainty, there is little compelling evidence for the concept of terminal differentiation except in organs in which differentiation is defined by cell death or loss of nuclei. The instability and plasticity of the differentiated state (Bissell 1981, Blau & Baltimore 1991) allow phenotypic evolution to occur over the lifetime of a cell, tissue, organ, and organism to ensure adaptability and survival. The differentiated phenotype accomplishes this while being both (*a*) robust (stable to minor perturbations; the breast almost never turns into a kidney in vivo) and (*b*) labile, or responsive to external influences. With regard to the latter characteristic, given the appropriate cues, a resting mammary gland can easily be coaxed into a spectacular reversible functional differentiation program during pregnancy, corneal epithelium can be induced to sprout feathers or hair (Coulombre & Coulombre 1971, Ferraris et al. 1994), and aggressive carcinoma cells can be tamed to form normal tissues by changing their microenvironment (Mintz & Illmensee 1975) or to revert to a normal phenotype simply by changing microenvironmental signaling (Weaver et al. 1997, 2002). The interactions between a cell and its surroundings thus determine its pattern of gene expression and resultant differentiated phenotype despite the fact that the blueprint of the genome does not change. Here we describe what we know about the process of tissue specificity from the point of view of the mammary gland (our experimental organism), but the fundamentals of the issues discussed extend far beyond this organ. In the end, the unit of functional differentiation is the organism itself.

## TISSUE ARCHITECTURE IS BOTH A CONSEQUENCE AND A CAUSE (THE END AND THE BEGINNING)

### Dynamic Reciprocity Redux

The structure of a tissue or organ is critical for its function. Loss of tissue architecture

is a prerequisite for, and one of the defining characteristics of, most cancers. Conversely, normal organ architecture can act as a powerful tumor suppressor, preventing malignant phenotypes even in cells stricken with gross genomic abnormalities (Mintz & Illmense 1975, Howlett et al. 1995, Weaver et al. 1997, Wang et al. 2002, Kirshner et al. 2003). But if organ function and homeostasis are driven by organ architecture, and if every cell in every organ carries the same genetic information, then how are tissue-specific form and function achieved? Elegant work by early developmental biologists, some of which is described below, inspired us to postulate that tissue-specific function is achieved by interactions between the cell and its surrounding extracellular matrix (ECM), a model dubbed dynamic reciprocity (Bissell et al. 1982). According to this model, the dynamic bidirectional cross talk from the ECM with the cell membrane (Bornstein et al. 1982) is extended to the broad realm of gene expression by connecting ECM–ECM receptor interactions to the cytoskeleton and to the nuclear matrix and chromatin and back again (reproduced in **Figure 1*a***). An important feature of this model was that it took the then-evolving work of the role of ECM in development as that of a possible scaffold to a view of ECM as an integral determinant of tissue specificity itself. Most importantly, the work of a number of laboratories has provided substantial evidence for the essential components of the model in the intervening years. Although the original depiction of dynamic reciprocity dealt mainly with the role of the ECM, the cellular microenvironment also clearly includes adhesive and soluble paracrine signals from neighboring cells, distant tissues, and systemic cues (see **Figure 1*b*** for updated model). As such, organ structure and consequently organ function are determined by the dynamic and reciprocal interactions between the organ's constituent tissues, the structure and function of which are determined by the dynamic and reciprocal interactions between the cells and ECM comprising a given tissue. And lest we forget, each organ is choreographed to function in a dynamic scenario with other organs and is of little use when removed from the greater context of the organism.

**ECM:** extracellular matrix

**Morphogenesis:** the process of development by which an organ achieves its final structure

## Tissue Interactions in Development

Every organ is composed of tissues derived from the embryonic germ layers: endoderm (which becomes epithelium of the lungs and digestive organs), mesoderm (which generates bone, muscle, and mesenchymal connective tissue), and ectoderm (which gives rise to the nervous system and epithelium of the skin and its derivatives, including the mammary gland). Epithelial and mesenchymal components interact during development to direct tissue morphogenesis (the physical creation of normal tissue architecture) and differentiation (acquisition of tissue-specific functions). That tissue development is not cell autonomous but is instead instructed by the surrounding environment was hypothesized as early as 1817 (Pander 1817). However, it was first demonstrated a century later by the elegant experiments of Ethel Browne, and Hans Spemann and Hilde Mangold, who used hydra and amphibian embryos, respectively (Browne 1909, Spemann 1918, Spemann & Mangold 1924). The famous organizer experiment showed that certain regions of the embryo could direct the development of adjacent groups of cells into specific tissues (Spemann 1918, Spemann & Mangold 1924).

These early studies preceded a flurry of work over the next 80 years, demonstrating in many systems that cells derived from the different germ layers carry on an extensive cross talk to direct tissue development. Studies of vertebrate skin (**Figure 2*a***) revealed that the identity, location, and pattern of development of ectodermal epidermal appendages (e.g., hair follicles in mammals and scales and feathers in birds) are determined by the dermis (a mesodermal derivative). Using tissue recombination techniques developed in the 1950s, Saunders and colleagues found that thigh mesoderm inserted

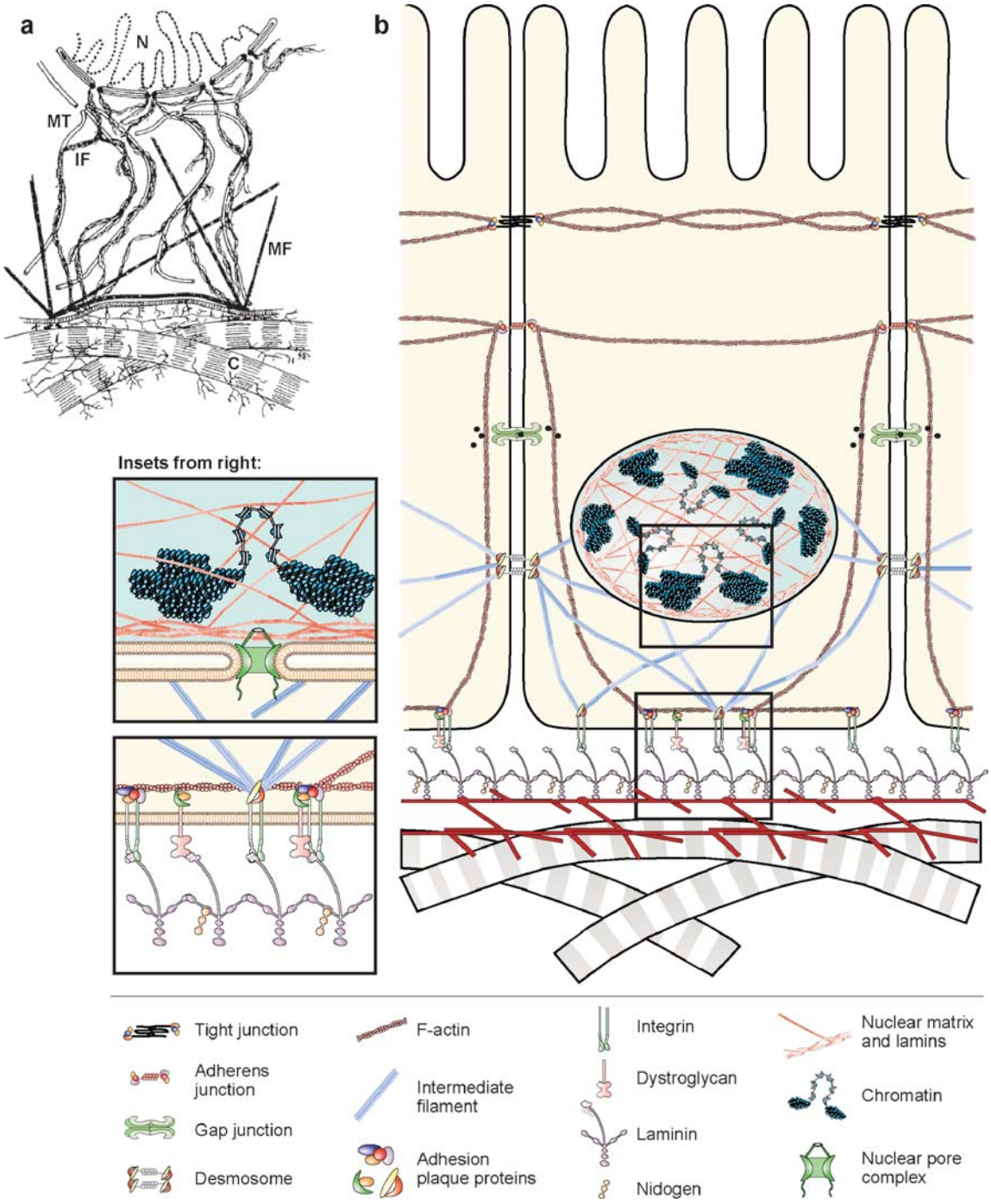


**Figure 1**

(*a*) The original model of dynamic reciprocity, or the minimum required unit for tissue-specific functions. N, nucleus; MT, microtubules; IF, intermediate filaments; MF, microfilaments; C, collagen. Reprinted from Bissell et al. (1982) with permission from Elsevier. (*b*) A more complete view of dynamic reciprocity.

beneath the ectoderm of an embryonic chick wing induced the wing to form leg feathers instead of flight feathers (Cairns & Saunders 1954, Saunders & Gasseling 1968). Chimeric feathers were occasionally found at the border of the graft site, demonstrating the specificity of the mesodermal signal. In similar studies recombining skin tissues from chick and duck, duck mesoderm instructed chick ectoderm to form feathers anatomically shaped like those of a duck; the converse was also true (Dhouailly 1967, 1970). In perhaps the most striking example, mesoderm from a mouse (which normally would induce mouse ectoderm to form hair follicles) was combined with corneal epithelium from a chick (which normally would become an appendage-free transparent surface), resulting in feather development (Coulombre & Coulombre 1971).

Ectoderm can play an instructive role during development as well. In vertebrates, the mesenchyme of the outgrowing limb is surrounded by a dorsal rim of ectoderm, the apical ectodermal ridge (AER). When the AER is removed, the limb fails to develop properly; when the AER covering the eventual wing is grafted onto the stump of a growing leg, the region develops wing parts (Saunders 1948). The mechanisms underlying induction by AER, skin mesoderm, and Spemann's organizer have been studied extensively (reviewed recently in Wolpert 1998, Capdevila & Izpisua Belmonte 2001, Niehrs 2004) and involve common paracrine signaling molecules, including members of the fibroblast growth factor, transforming growth factor (TGF)-β, Wnt, and hedgehog families.

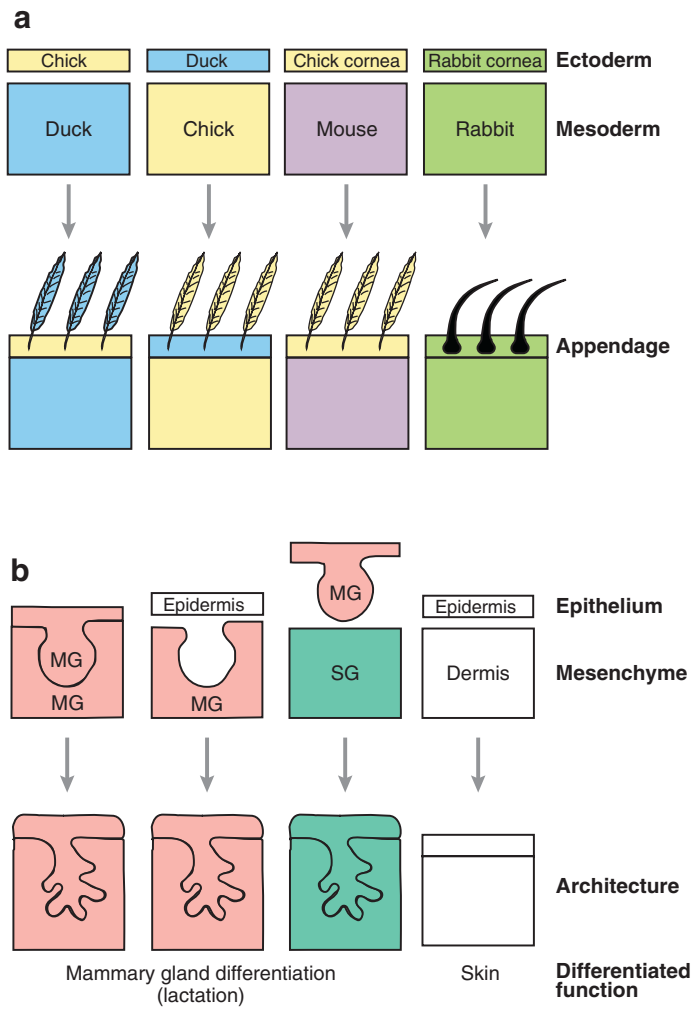


**Figure 2**

The dramatic effect of tissue-tissue interactions. (*a*) Embryonic ectoderm/mesoderm recombination experiments determined that the identity of the mesoderm dictated the identity of the ectodermal appendage. (*b*) Epithelial/mesenchymal recombination experiments determined that the identity of the mesenchyme dictated the architecture of the developing epithelium. When mammary gland (MG) epithelium is recombined with salivary gland (SG) mesenchyme, the resulting structure can still produce milk, although the epithelial tree resembles a salivary gland. Panel *b* adapted from Parmar & Cunha (2004).

## The Impressionable Epithelium

Some of the clearest examples of the importance of epithelial-mesenchymal interactions in morphogenesis and differentiation have come from recombination experiments using isolated tissues from the mammary gland and other organs (**Figure 2*b***). Whereas mammary epithelium recombined with mammary mesenchyme develops a typical mammary tree, recombination with salivary gland mesenchyme generates structures resembling the salivary epithelial tree (Kratochwil 1969, Sakakura et al. 1976). Conversely, mammary mesenchyme can induce epithelial cells from other tissues to build a lactation-competent

**Table 1 Epithelial-mesenchymal interactions in the mammary gland**

| | Signaling by stroma | | Signaling by epithelium | |
|---|---|---|---|---|
| | **Stromal ligand/cue** | **Epithelial receptor** | **Epithelial ligand/cue** | **Stromal receptor** |
| During ductal development/puberty | HGF<br>IGF-I<br>Activin/inhibin B<br>Epimorphin<br>MMP-2, -3, -9, -11 | cMet<br>IGF-I receptor<br>Activin receptors<br>Unknown<br>N/a | Amphiregulin<br>TGF-β<br>PTHrP | EGFR (ErbB1)<br>TGFβR-I, -II<br>PTHrP receptor |
| During alveolar development/ pregnancy | Neuregulin<br>Activin/inhibin B<br>KGF (FGF-7)<br>Epimorphin<br>MMP-3 | ErbB3/ErbB4<br>Activin receptors<br>FGFR2-IIIb<br>Unknown<br>N/a | | |



**Mesenchyme:** mass of connective tissue, mainly derived from mesoderm, in embryonic and developing organs that usually develops into the stroma

gland (Cunha et al. 1995). These experiments demonstrated that even adult cells retain a capacity for alternative modes of morphogenesis and differentiation. The importance of reciprocal interactions between epithelium and mesenchyme and the identification of the molecular mediators have now been demonstrated for several organs, including the lung, kidney, prostate, and salivary and mammary glands (reviewed in Hieda & Nakanishi 1997, Cardoso 2001, Marker et al. 2003, Parmar & Cunha 2004, Yu et al. 2004). The molecular players involved in epithelial-mesenchymal interactions during mammary gland development are detailed in **Table 1**; similar roles for many of these molecules have been found in the development of other organs.

Tissue interactions are thus a major source of information regulating tissue-specific activation of genes leading to the proper development of cells, tissues, and organs (Wessells 1977). As an example, **Figure 3** depicts reciprocal interactions between the cells and tissues that comprise the adult mammary gland and between the mammary gland and other organs. As alluded to above and discussed in depth below (see section on Three-Dimensional Models of Mammary Gland Development), the morphogenesis of the mammary epithelium is regulated by its interactions with mesenchymal cells. During branching morphogenesis of mammary and other organs, nerves, blood vessels, and epithelium grow out simultaneously in intimately interacting trees (Coughlin 1975, Gebb & Shannon 2000). The details of these presumed communications have yet to be uncovered for the mammary gland, but in skin, peripheral nerves determine the pattern of arterial branching by stimulating localized secretion of vascular endothelial growth factor (VEGF) (Mukouyama et al. 2002). Additionally, the kinetics of development and functional differentiation (milk synthesis and secretion) are controlled by influences external to the epithelium, including pituitary and ovarian hormones, and mechanical cues from suckling at the nipple, which activates contraction of the myoepithelial cells.

## Of Terminal Differentiation and Molecular Vitalism

In this age of genomics and gene expression arrays, one could easily accept the argument that a cell's status (for example, its identity and the identity of the tissue and organ in which it resides) could be inferred mainly by examining the genes that it expresses. Although this may very well prove to be true, it is a fallacy to argue that therefore it is the genes themselves that determine and regulate the pattern of gene expression. Additionally, are the genes expressed the sole determinant of the status of a cell or how it may behave? The data from tissue recombination studies suggest that even

differentiated cells retain a high degree of flexibility, or as Marc Kirschner and colleagues described so eloquently, an "interconvertible multi-statedness is a key aspect of multicellular self-organization" (Kirschner et al. 2000). This flexibility is apparent during tissue regeneration and repair and to a remarkable degree in organisms, such as the newt, that can regenerate entire organs and limbs even in the adult animal. That a differentiated cell (meaning, for example, a cell that has become a hepatocyte and functions within the context of the liver) can even respond to cues that direct the development of a different tissue to express muscle myosin (Chiu & Blau 1984, Blau et al. 1985) should have dispelled the notion that the process of differentiation locks cells into a particular fate without recourse. Indeed, cultured cells that invariably lose their differentiated phenotypes when grown in a petri dish can be induced to form both normal and diseased tissue structures when returned to the appropriate environment in vivo (DeOme et al. 1959, Daniel & DeOme 1965). Similarly, cells in culture can regain their differentiated phenotypes if the microenvironment of the culture vessel is tailored to mimic the cell's normal microenvironment in vivo (reviewed in Bissell 1981 and below).

## THREE-DIMENSIONAL MODELS OF MAMMARY GLAND DEVELOPMENT: RATIONALE AND EXAMPLES

### The Structure of the Human Breast

The mammary gland is an excellent example of an organ, the development and differentiation of which require dynamic and reciprocal signaling between cells and their (micro)environment. Unlike other organs, the majority of mammary gland development occurs postnatally during puberty. In females, a surge of steroid hormones induces the anlage (the mammary ductal rudiment present at birth) to undergo a burst of branching morphogenesis. The mammary gland is

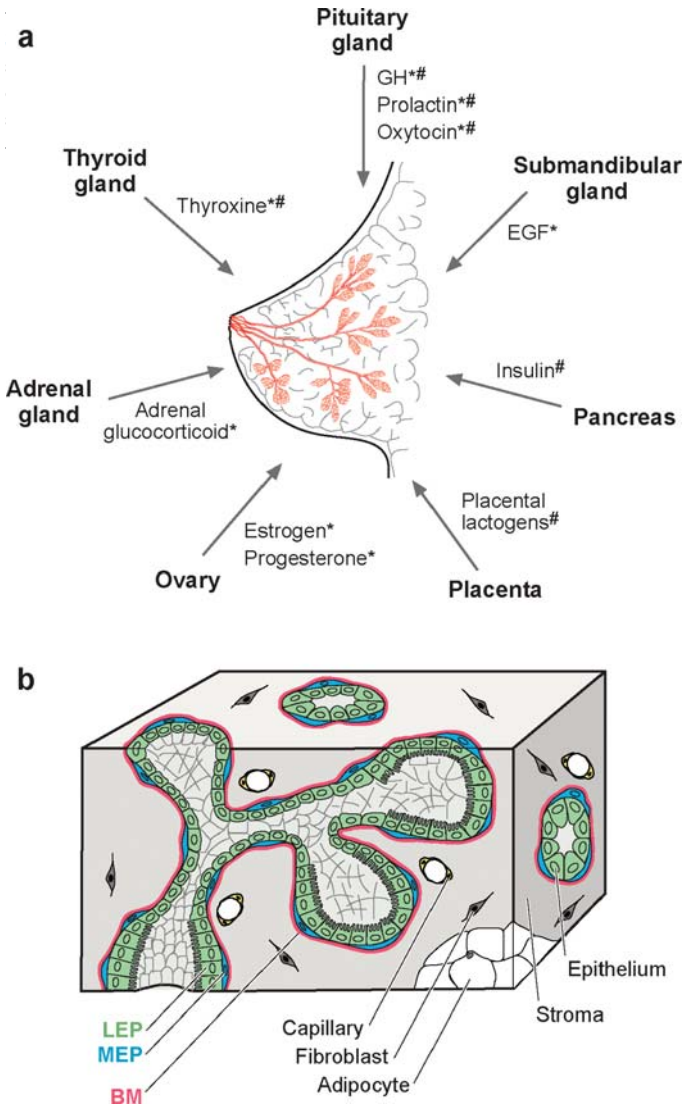


**Figure 3**

The structure and function of the mammary gland are influenced by communication with distant organs and between constituent tissues. (*a*) The human breast is a bilayered epithelial ductal tree (*pink*) embedded in a complex stroma. Signals released from distant organs influence ductal and acinar morphogenesis during puberty (*) and pregnancy (#) (reviewed in Hovey et al. 2002). (*b*) The epithelium consists of a layer of luminal epithelial cells (LEP) surrounded by myoepithelial cells (MEP) and basement membrane (BM). The epithelium is surrounded by a fibrous stromal compartment and adjacent fatty stroma. Molecular details of epithelial-mesenchymal interactions are described in **Table 1**.

**BM:** basement membrane

**lrECM:** laminin-rich ECM

**TDLU:** terminal ductal lobular unit

**EMT:** epithelial-to-mesenchymal transition

**MMP:** matrix metalloproteinase



composed of two tissue compartments, the ectodermally derived epithelium and the mesodermally derived stroma (depicted schematically in **Figure 3**). The bilayered epithelial tree consists of a central layer of luminal epithelial cells surrounded by a layer of myoepithelial cells and basement membrane (BM), a specialized laminin-rich form of ECM (lrECM). In humans, the epithelium (both luminal and myoepithelial) is surrounded by a loose intralobular connective tissue stroma and a denser interlobular stroma, which together account for 80% of the volume of the resting breast and house nerves, blood vessels, and lymphatics (Drife 1986). The ducts terminate in lobular structures known as terminal ductal lobular units (TDLUs), which give rise to alveolar buds during pregnancy that become secretory alveoli during lactation. Luminal epithelium is induced during lactation to produce and vectorially secrete milk into the ducts; milk is squeezed through the mammary tree to its opening at the nipple by concerted contraction of myoepithelial cells induced by suckling. Once lactation is terminated by cessation of suckling, the gland remodels during involution by the concerted action of hormones, metalloproteinases, and molecules involved in apoptosis (Talhouk et al. 1991, 1992; for a recent review, see Hennighausen & Robinson 2005).

## Signaling by the Microenvironment

Interactions between luminal epithelial cells, ECM and its remodeling enzymes, and the other cells of the gland are critical for development and differentiation (Fata et al. 2004, Parmar & Cunha 2004). Myoepithelial cells secrete laminin-1 to build the BM that surrounds the epithelial compartment (Gudjonsson et al. 2002), direct the polarization of luminal epithelial cells (Runswick et al. 2001, Gudjonsson et al. 2002), and regulate morphogenesis of the ductal tree (Niranjan et al. 1995). Loss of these activities correlates with breakdown of normal mammary architecture and leads to tumor progression (reviewed in Adriance et al. 2005). During branching morphogenesis at puberty (Witty et al. 1995, Fata et al. 1999, Wiseman et al. 2003), and later during involution of the gland upon weaning (Talhouk et al. 1992, Lund et al. 1996), extensive breakdown and remodeling of the ECM occur via precise expression/activation/inhibition of matrix-degrading enzymes, especially members of the matrix metalloproteinase (MMP) family. Inappropriate expression of MMPs causes breakdown of the BM, disrupting functional differentiation (milk protein expression) of luminal epithelial cells (Sympson et al. 1994, Witty et al. 1995) and, in the case of MMP-3, leading to epithelial-to-mesenchymal transition (EMT), apoptotic cell death, genomic instability, induction of a reactive fibrotic stroma, and eventually tumor formation (Sympson et al. 1995; Alexander et al. 1996; Lochter et al. 1997; Thomasset et al. 1998; Sternlicht et al. 1999, 2000; Radisky et al. 2005). One mechanism by which destruction of BM leads to EMT and genomic instability is through increased levels of cellular reactive oxygen species, which upregulate expression of certain transcription factors and cause oxidative DNA damage (Radisky et al. 2005).

Proper development of the ductal tree depends on permissive and instructive cues from the stromal compartment. For example, both epithelial and stromal cells express estrogen receptor (ER)-α, and mammary glands from ER-α-knockout mice have a rudimentary underdeveloped ductal tree (Bocchinfuso & Korach 1997). Experiments recombining epithelium and stroma from wild-type and ER-α-knockout mice demonstrated that estrogen signaling is required in stromal cells during ductal morphogenesis (Cunha et al. 1997). Further experiments in culture revealed that, in response to estrogen, stromal fibroblasts produce hepatocyte growth factor (HGF), which acts in a paracrine role to induce growth of the epithelial tree (Zhang et al. 2002a). Reciprocal signaling from

epithelium to the stroma is also required for the development of the gland. Epidermal growth factor receptor (EGFR) is required in the stromal compartment (Wiesen et al. 1999). The EGFR ligand, amphiregulin, is expressed on and cleaved from the surface of the epithelium by the cell-surface sheddase ADAM (a disintegrin and metalloproteinase)-17, presumably in response to estrogen signaling (Sternlicht et al. 2005). Consequently, mammary development is impaired in mice expressing signaling-defective EGFR (Fowler et al. 1995, Xie et al. 1997, Sebastian et al. 1998). These positive signals are balanced by negative cues, including TGF-β. Members of the TGF-β superfamily and their receptors are expressed throughout development of the gland (reviewed in Daniel et al. 2001, Serra & Crowley 2005). TGF-β in particular inhibits branching morphogenesis during puberty (Silberstein & Daniel 1987, Robinson et al. 1991, Pierce et al. 1993), blocks formation of alveoli and secretion of milk during pregnancy (Jhappan et al. 1993, Kordon et al. 1995, Siegel et al. 2003), and promotes apoptosis during involution (Nguyen & Pollard 2000, Gorska et al. 2003, Bailey et al. 2004).

The mesenchymal compartment also expresses morphogens, including epimorphin (Hirai et al. 1998, 2001; Simian et al. 2001) and members of the Wnt and notch families, that guide the development of the epithelial tree (Uyttendaele et al. 1998). That overexpression of epimorphin in the mammary gland leads to tumor development (Bascom et al. 2005) highlights the stroma's importance in regulating conversion to the malignant phenotype, a concept introduced more than 100 years ago (Paget 1889). Normal stroma has tumor-suppressive properties, in contrast to stroma derived from breast cancer. Embryonic mammary mesenchyme can induce differentiation of mammary tumors (DeCosse et al. 1973). Conversely, human breast cancer xenografts produce significantly faster growing tumors when the cells are mixed with carcinoma-derived fibroblasts than when the cells are mixed with normal fibroblasts (Camps et al. 1990, van Roozendaal et al. 1996, Dong-Le Bourhis et al. 1997) or when they are injected into a previously irradiated stroma (Barcellos-Hoff & Ravani 2000). The latter effect is apparently due to irradiation-induced activation of TGF-β, which is the culprit in wound-induced tumors (Sieweke et al. 1990) and is known to lead to a fibrotic response in abnormal microenvironments by increasing synthesis of ECM molecules such as collagen I (Ehrhart et al. 1997). Increased tissue stiffness itself can promote malignant transformation by leading to deregulated integrin signaling (Paszek et al. 2005), and patients with such fibrotic lesions have a poor prognosis (Colpaert et al. 2001).

Breast carcinomas consist not only of the aberrant epithelial cells and stroma but also recruited blood vessels, activated fibroblasts, and infiltrating macrophages, lymphocytes, and leukocytes. Growing evidence points to recruitment of macrophages as important for breast tumor progression, with macrophage infiltration correlating with a poor prognosis (Leek et al. 1996, Goswami et al. 2005). Finally, alterations in the stroma are not solely due to changes in the constituent population of cells or deposition of ECM because stroma associated with breast tumors contains both genetic and epigenetic alterations (Deng et al. 1996, Washington et al. 2000, Allinen et al. 2004, Hu et al. 2005), and stromal fibroblasts in which the TGF-β type II receptor is inactivated stimulate the development of tumors in the adjacent epithelium (Bhowmick et al. 2004, Radisky & Bissell 2004). Clearly, the context in which an epithelial cell receives an oncogenic insult plays a large role in whether or not that cell generates a frank tumor, as shown in a number of earlier studies (for a review, see Kenny & Bissell 2003).

## Organotypic Culture Models to Study Form, Function, and Dysfunction

Many of the details of microenvironmental signaling in the mammary gland have

been uncovered using three-dimensional (3D) culture models (for historical overviews, see Bissell et al. 2003, 2005; Nelson & Bissell 2005). Differentiated mammary epithelial cell structure and function can be reproduced in culture when cells are given an appropriate microenvironment that recapitulates aspects of the above-described tissue structure. When grown on plastic substrata, human and rodent mammary epithelial cells flatten out and fail to respond to lactogenic cues; that is, they "forget" their mammary phenotype. However, when grown within a malleable lrECM, these same cells will assemble into polarized 3D acinar structures that resemble alveoli in vivo (Emerman & Pitelka 1977, Lee et al. 1985, Barcellos-Hoff et al. 1989, Aggeler et al. 1991). Cells that are not attached to BM undergo apoptosis (Boudreau et al. 1995), and apoptosis of cells in the center of the structures leads to the formation of hollow lumina (Blatchford et al. 1999, Debnath et al. 2002, Mills et al. 2004), a process similar to canalization of the ducts in vivo (Humphreys et al. 1996). When stimulated with lactogenic hormones, cultured acini of rodent epithelial cells express and secrete milk proteins into the central lumina (Emerman & Pitelka 1977; Lee et al. 1984, 1985; Streuli et al. 1995b). The binding of laminin-1 to integrin and other ECM receptors, now shown to include dystroglycan (M.L. Wier, M.L. Oppizzi, M.D. Henry, A. Onishi, K.P. Campbell, et al., manuscript submitted), causes changes in both cell shape and biochemical signaling to induce functional differentiation (Streuli et al. 1991, 1995b; Roskelley et al. 1994; Muschler et al. 1999). Even though milk appears to be expressed upon parturition with all protein constituents simultaneously, 3D culture studies have revealed that there is specificity in the regulation by microenvironmental context: Lactoferrin expression only requires cell rounding and β-casein can be expressed by single, rounded cells in contact with laminin, whereas the expression of whey acidic protein (WAP) requires formation of the polarized acinus (reviewed in Roskelley et al. 1995).

In addition to illuminating the processes of acinus formation and milk protein secretion, 3D culture models have been highly successful in recapitulating the epithelial remodeling and invasion central to the branching morphogenesis that builds the initial epithelial tree during puberty. Primary epithelial organoids or mammary epithelial cell lines cultured within gels of collagen I or lrECM can be induced to form branching structures by coculture with stromal fibroblasts or by exogenous addition of growth factors, such as HGF or epidermal growth factor (EGF) (Brinkmann et al. 1995, Soriano et al. 1995, Yang et al. 1995, Hirai et al. 1998, Niemann et al. 1998, Simian et al. 2001), or of cytokines, such as members of the tumor necrosis factor (TNF)-α family (Lee et al. 2000, Michaelson et al. 2005). Blocking either MMP activity or cell binding to epimorphin prevents branching (Hirai et al. 1998, Lee et al. 2000, Simian et al. 2001, Michaelson et al. 2005). To initiate a branch, epithelial cells must transiently loosen their interactions with neighboring cells and invade the surrounding ECM. Culture models of mammary and kidney epithelial branching have revealed that cells at the leading edge of branches undergo a transient or partial EMT (O'Brien et al. 2004; C.S. Chen, C.M. Nelson, S. Bennett, C. Gilles, Y. Hirai, et al., manuscript in submission)—one of many developmental processes frequently hijacked by cancer cells—which requires coordinate signaling from growth factors, MMPs, and epimorphin.

Recreating the microenvironment in culture also allows one to distinguish clearly between cells that do and do not differentiate (such as normal and tumorigenic breast cells), something difficult to achieve in traditional two-dimensional cultures. Whereas normal cells form polarized growth-arrested acini when cultured in 3D lrECM (Barcellos-Hoff et al. 1989), breast cancer cell lines or primary cells derived from carcinomas form highly disorganized and proliferative colonies reminiscent of tumors (Petersen et al. 1992, Weaver et al. 1995). Antagonizing one

or more of the many pathways that are dysregulated in tumor cells causes them to functionally revert to a normal phenotype: The cells stop growing, form polarized acini, and are less tumorigenic when injected into nude mice (Howlett et al. 1995, Hirschi et al. 1996, Weaver et al. 1997, Wang et al. 1998, Kirshner et al. 2003, Liu et al. 2004, Park et al. 2006). Additionally, the activation levels of the other signaling pathways normalize to levels seen in nontumorigenic cells (for a review, see Bissell et al. 2005). These results demonstrate that tumorigenicity is context dependent, that tissue structure can be dominant over genotype, and that differentiation therapy, a concept used in treating some forms of leukemia, is a potentially powerful strategy for cancer therapy.

## TISSUE SPECIFICITY IN THE MAMMARY GLAND AND BEYOND: CONTEXT IS ALL

### From ECM to ECM-Response Elements

In the presence of a malleable laminin-rich substratum, mammary epithelial cells round up, organize into acinar structures, hollow out to form a central lumen, and secrete milk proteins, including β-casein, in response to lactogenic hormones. The laminin-induced expression of β-casein involves activation of an ECM-response element (ECM-RE) in the promoter of the β-casein gene (Schmidhauser et al. 1990, Schmidhauser et al. 1992, Myers et al. 1999) by β1-integrin-induced phosphorylation of the prolactin receptor, thus allowing prolactin to regulate the DNA-binding activity of the Stat5 transcription factor (Streuli et al. 1995a, Edwards et al. 1998). ECM-REs have been found in the promoter regions of several proteins, including those of αs1-casein (Jolivet et al. 2005), albumin (Liu et al. 1991), and TGF-β, which is regulated negatively by laminin (Streuli et al. 1993). Given that a multitude of ECM molecules is part of what comprises the microenvironment, we can imagine that the family of ECM-REs will be refined in the future to include, for example, laminin-response element, collagen-response element, and various combinations thereof. ECM also regulates the expression of tissue-specific transcription factors, such as mammary gland factor (MGF, or Stat5a) (Schmitt-Ney et al. 1991), which can thereby transduce context-dependent information indirectly by binding to the promoter regions of milk protein genes (Groner & Gouilleux 1995).

**ECM-RE:** ECM-response element

ECM-induced formation of the polarized acinus affects signaling between epithelial cells. In response to laminin, mammary epithelial cells upregulate expression of several of the connexin gap junction proteins, enhancing gap junctional intercellular communication (GJIC) (El-Sabban et al. 2003). Inhibiting GJIC downregulates β-casein expression. That loss of connexin expression leads to and correlates with tumor progression and that reexpression of connexins can inhibit the metastatic phenotype highlight the importance of cell-cell communication in guiding and responding to tissue architecture (Carystinos et al. 2001). Indeed, disrupting tight junctions prevents the establishment of tissue polarity and disrupts the structure of already polarized cells, leading to neoplastic growth (reviewed in Itoh & Bissell 2003).

Aside from inducing signal transduction through integrins and determining tissue morphology, the microenvironment also affects the structure of the nucleus. Histone acetylation promotes chromosome decondensation and unfolding, increasing the accessibility to transcription factors and other regulatory machinery, thereby enhancing transcription (Jenuwein & Allis 2001). Activation of the ECM-RE in the promoter of the β-casein gene can be modulated by altering the organization of histones (Myers et al. 1998), and addition of laminin induces histone deacetylation in mammary epithelial cell lines (Pujuguet et al. 2001). Recent experiments have demonstrated that cell rounding by itself (independent of cell-ECM interactions)

leads to histone modifications (J. Le Beyec, R. Xu, S.Y. Moonlee, C.M. Nelson, A. Rizki, and M.J. Bissell, unpublished data). Because the cytoskeleton appears to physically connect the ECM to the nucleus through ECM receptors (Maniotis et al. 1997), and because destruction of ECM by MMPs leads to genomic instability through alternative splicing of the Rac1 transcript (Radisky et al. 2005), it is tempting to speculate that the effects due to changes in cell morphology are transmitted to the nucleus through the cytoskeleton. Taken together, these data support and expand dynamic reciprocity (**Figure 1*a*,*b***), whereby tissue specificity is determined and maintained by interactions of adhesion receptors with surrounding ECM and neighboring cells. These interactions activate downstream signaling pathways, in conjunction with altering cytoskeletal structure and cell and nuclear morphology, to modulate binding of transcription factors to the microenvironment-specific response elements of tissue-specific genes. The resulting changes in gene expression modify a panoply of signaling proteins produced by the cell, including ECM proteins and tissue-specific transcription factors, cementing the organ-specific phenotype.

## Tissue Specificity Throughout Evolution

If context directs development, then do organs that develop similar structures do so using similar contextual cues? The answer, at least for the branched organs of placental mammals, appears to be a qualified "yes." The pancreas, lung, kidney, prostate, and salivary and mammary glands all develop by branching morphogenesis, driven by epithelial-mesenchymal interactions involving stimulatory signaling in part from HGF and EGF, balanced by inhibitory signaling from members of the TGF-β family, and regulated by ECM and MMPs (reviewed in Davies 2002). This conservation of contextual signaling was first glimpsed in the tissue recombination experiments of the 1960s, discussed above (see section on Tissue Architecture Is Both a Consequence and a Cause). Interestingly, the epithelium in these organs is initially derived from different germ layers: endoderm in the pancreas and lung, mesoderm in the kidney, and ectoderm in the mammary gland. However, there are also major differences in the contexts under which each of these organs develops, which likely plays a role in the final tissue-specific architecture and function achieved. The pattern of branching of the lung is determined by embryonic patterning cues (Chuang & McMahon 2003), the kidney has its own growth factor [glial cell–derived neurotrophic factor (GDNF)], and the mammary gland develops uniquely in the context of puberty.

Although the mammary gland is a relatively recent evolutionary acquisition (Oftedal 2002), the similarities between its development and that of other, more ancient organs (such as the pancreas, which is present as a branched structure even in cartilaginous fish, of which the last common ancestor to mammals was 450 Mya) suggest that some of the above-described mechanisms for directing tissue specificity may be conserved (last reviewed in Ashkenas et al. 1996). Indeed, homologs of ECM proteins and integrins are present in many invertebrates. The nematode worm *Caenorhabditis elegans* expresses collagens and a β1-integrin homolog, $\beta_{pat-3}$; mutations in the collagen IV homologs emb-9 and let-2 are embryonic lethal, suggesting the importance of BM in worm development (Kramer 1994). The fly *Drosophila melanogaster* expresses laminins, dystroglycan, and a number of α- and β-integrins, and similar to the mammary gland, dystroglycan is required for generation of apico-basal polarity in Drosophila epithelial cells (Deng et al. 2003). Hydra express laminins, collagens, MMPs, and a putative β1-integrin, which are required for proper epithelial morphogenesis during head and tentacle regeneration (Shimizu et al. 2002, Zhang et al. 2002b). Even the slime mold *Dictyostelium discoideum* expresses ECM during its multicellular slug phase

and stalk development, which is regulated by a Stat transcription factor homolog (Shimada et al. 2004). ECM-REs are also evolutionarily conserved, at least functionally, if not in nucleotide sequence: Sea urchin embryonic development requires collagen-induced activation of a short promoter element in the LpS1 gene (Seid et al. 1997). Because cytoskeleton is, in general, conserved through different phyla (Muller et al. 2005), it is likely that cell and tissue context play an analogous role in the development, differentiation, and homeostasis of many organisms.

## INTEGRATION

A fundamental property of all known (and therefore, presumably, successful) forms of life is the ability to adapt to changes in both the environment external to the organism and the internal (micro)environment. Terminal change—an inability to adapt—in all dynamic systems leads to equilibrium, which for living things is death. Dynamic reciprocity, then, is scalable both in time and space and is a mechanism by which single cells within tissues maintain homeostasis in spite of an uncertain environment over the organism's lifetime. Tissue-specific context is thus important not only for development and differentiation but also as a protective mechanism against cancer and other diseases. However, as much as we might wish otherwise, tissue context is not static even in the adult, succumbing eventually to the effects of living: reactive oxygen species, carcinogens, diet, shrinking telomeres—in sum, the effects of aging (Hasty et al. 2003). The context of an old breast is not the same as that of a young breast. As menopause approaches, epithelial cells die off, the stromal compartment alters, the entire morphology of the organ changes. It is instructive to combine our vast knowledge of developmental biology with emerging concepts in tissue specificity so as to generate an integrated understanding of development, homeostasis, cancer, and aging.

The essence of what we have laid out here is that the integration of signaling hangs on the structure of an organ, for structure has information, a kind of information distinct from the genomic blueprint of the cell. When one considers all of the signaling pathways involved in differentiation, the complexity is staggering. There is clearly more than one way of integrating the same combination of signals into a phenotype (Bissell et al. 2003); this is precisely why development is so miraculously robust.

## FUTURE DIRECTIONS: DECODING THE LANGUAGE OF FORM

Organ architecture is thus both a consequence and a cause for development, differentiation, and homeostasis. But how does the architecture of an organ (or tissue, or cell) make itself heard? We understand something about the alphabet (ECM, receptors, cytoskeleton, nuclear matrix, chromatin) and even less about the rules of grammar that turn random words into commands (activation of tissue-specific response elements). We believe that decoding this language requires abandoning the currently fashionable "molecule-centric" style of inquiry and adopting a more interdisciplinary approach that takes into account dynamic changes, spatial segregation of events, and tissue architecture.

### SUMMARY POINTS

1. Development, differentiation, and homeostasis are controlled by cell-cell interactions, cell-ECM interactions, ECM-degrading enzymes, and soluble cues (hormones, cytokines, and growth factors).

2. Malignant phenotype can be reverted without changing genotype. Thus phenotype can be dominant over genotype.
3. Signaling pathways are context dependent.
4. Maintenance of homeostasis requires maintenance of form.



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