

**Of extracellular matrix, scaffolds, and signaling:
Tissue architecture regulates development, homeostasis, and cancer**

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Abstract

The microenvironment surrounding cells influences gene expression, such that a cell's behavior is largely determined by its interactions with the extracellular matrix, neighboring cells, and soluble cues released locally or by distant tissues. We describe the essential role of context and organ structure in directing mammary gland development and differentiated function, and in determining 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 piano keys: while essential, it is the context that makes the music.

Glossary

Mesenchyme: mass of connective tissue, mainly derived from mesoderm, in embryonic and developing organs which will develop into the stroma. Mesenchyme directs the morphogenesis of epithelial cells, and in turn is induced to differentiate by the epithelium.

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

Morphogenesis: (from the Greek *morphE* form and *gignesthai* to be born) the process of development by which a tissue or organ achieves its final structure. Morphogenesis involves changes in the shape, growth, division, migration, and death of individual cells within tissues.

Stroma: (from the Greek *stornyai* to spread out) the supporting connective tissue scaffold of an organ.

Acronyms

3D, three-dimension(al)

ADAM, a disintegrin and metalloproteinase

AER, apical ectodermal ridge

BM, basement membrane

ECM, extracellular matrix

ECM-RE, ECM-response element

EGF, epidermal growth factor

EGFR, epidermal growth factor receptor

EMT, epithelial-to-mesenchymal transition

ER- α , estrogen receptor- α

GDNF, glial cell-derived neurotrophic factor

GJIC, gap junctional intercellular communication

HGF, hepatocyte growth factor

lrECM, laminin-rich ECM

MGF, mammary gland factor

MMP, matrix metalloproteinase

TDLU, terminal ductal lobular unit

TGF- β , transforming growth factor- β

WAP, whey acidic protein

VEGF, vascular endothelial growth factor

I. INTRODUCTION

The function of an organ relies upon its constituent cell types and its overall organization. It is the obvious uniqueness of this structure that distinguishes 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: all this, despite the fact that they share an identical genome. However, while “tissue specificity” is a fact, there is little evidence for “terminal differentiation”, except in organs where 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 robust (stable to minor perturbations: the breast almost never turns into a kidney *in vivo*) and labile [responsive to external influences: given the appropriate cues, a resting mammary gland can easily be coaxed into a spectacular reversible 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)]. The interactions between a cell and its surroundings thus determine its pattern of gene expression and resultant differentiated phenotype. Here we describe 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.

II. 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 phenotype even in cells stricken with gross genomic abnormalities (Howlett et al 1995, Weaver et al 1997). 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 in the following sections, 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): extending the dynamic bidirectional crosstalk from the ECM with the cell membrane (Bornstein et al 1982) 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 as Figure 1A). An important feature of this model was that it took the then evolving work in the role of ECM in development as a possible scaffold to ECM as an extension of the tissue. Most importantly, ECM was viewed as an instructional entity crucial for attaining cell- and tissue-specificity and homeostasis. Although the original depiction of dynamic reciprocity dealt mainly with the role of the ECM, the cellular microenvironment also

includes adhesive and soluble paracrine signals from neighboring cells, distant tissues, and systemic cues (updated as Figure 1B). That being the case, organ structure (and consequently, organ function) is determined by the dynamic and reciprocal interactions between its constituent tissues, the structure and function of which are determined by the dynamic and reciprocal interactions between the cells comprising a given tissue. And, lest we forget, each organ is choreographed to function in a dynamic scenario with other organs and is, in and of itself, of little use when removed from the greater context of the organism.

Tissue interactions in development

Every organ is comprised 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), but first demonstrated a century later by the elegant experiments of Ethel Browne, Hans Spemann and Hilde Mangold using 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 following 80 years demonstrating in many systems that cells derived from the different germ layers carry on an extensive crosstalk to direct tissue development. Studies of vertebrate skin (Figure 2A) 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 beneath the ectoderm of an embryonic chick wing would induce 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 was found to instruct chick ectoderm to form feathers anatomically shaped like those of a duck; the converse was also found to be true (Dhouailly 1967, Dhouailly 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 the development of feathers (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.

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 2B). 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 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, has now been demonstrated for several organs, including the lung, kidney, prostate, 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 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 proper development of cells, tissues, and organs (Wessells 1977). As an example, Figure 3 depicts reciprocal interactions amongst 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 in Part III, 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)(Mukoyama 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 the status of a cell (its identity, the identity of the tissue and organ in which

it resides, etc.) could be inferred mainly by examining the genes that it expresses.

Whereas this may very well prove to be true, it is a fallacy of logic 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 described so eloquently by Marc Kirschner and colleagues: an “interconvertible multi-statedness is a key aspect of multicellular self-organization” (Kirschner et al 2000). This flexibility is clearly 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 decided it is epidermal and functions within the context of the skin) can even respond to cues that direct the development of a different tissue to form a mammary gland 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 (Bissell 1981) and in the following sections].

III. 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 comprised of two tissue compartments, the ectodermally-derived epithelium and the mesodermally-derived stroma, depicted schematically in Figure 3. The epithelium is a bi-layered ductal tree, consisting 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. 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 of the nipple. 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, Talhouk et al 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, Simian et al 2001, 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 occurs 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 (Simpson 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 (Simpson et al 1995, Alexander et al 1996, Lochter et al 1997, Thomasset et al 1998, Sternlicht et al 1999, Sternlicht et al 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 as well as cause oxidative DNA damage (Radisky et al 2005).

Proper development of the ductal tree relies 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)], with TGF- β in particular inhibiting branching morphogenesis during puberty (Silberstein & Daniel 1987, Robinson et al 1991, Pierce et al 1993), blocking formation of alveoli and secretion of milk during pregnancy (Jhappan et al 1993, Kordon et al 1995, Siegel et al 2003), and promoting apoptosis during involution (Nguyen & Pollard 2000, Gorska et al 2003, Bailey et al 2004).

The mesenchymal compartment also expresses morphogens, including epimorphin and members of the Wnt and notch families, that guide the development of the epithelial tree (Hirai et al 1998, Uyttendaele et al 1998, Hirai et al 2001, Simian et al 2001). That overexpression of epimorphin in the mammary gland leads to the development of tumors (Bascom et al 2005) highlights the importance of the stroma in regulating conversion to the malignant phenotype, a concept introduced over 100 years ago (Paget 1889). Normal stroma has tumor-suppressive properties compared 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 with normal fibroblasts (Camps et al 1990, van Roozendaal et al 1996, Dong-Le Bourhis et al 1997), or when the cancer cells 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 was shown to be 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, since stroma associated with breast tumors has been found to contain 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). 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 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 a historical overview, see (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 laminin-rich ECM (lrECM), these same cells will assemble into polarized 3D acinar structures that resemble alveoli in vivo (Emerman & Pitelka 1977, 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 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, Lee et al 1985, Streuli et al 1995b). Laminin-1 binding to integrin and non-integrin cell surface receptors (now shown to include dystroglycan-1; Wier, Muschler et al, submitted), causes both a change in cell shape and biochemical signaling to induce functional differentiation (Streuli et al 1991, Roskelley et al 1994, Streuli et al 1995b, 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, 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 acinus formation and milk protein secretion, 3D culture models have been highly successful in recapitulating the epithelial remodeling and invasion central to 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 co-culture 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 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, Chen et al 2006)—one of many developmental processes frequently hijacked by cancer cells—which require 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 between normal and tumorigenic breast cells), something difficult to achieve in traditional two-dimensional culture. Whereas normal cells form polarized growth-arrested acini when cultured in 3D lrECM, breast cancer cell lines or 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 (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 non-tumorigenic cells [for review see (Bissell et al 2005)]. These results demonstrate that tumorigenicity is context dependent, that tissue structure can be dominant over genotype, and that recreating normal tissue context is a potentially powerful strategy for cancer therapy.

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

From ECM to ECM-response elements

In the presence of a malleable laminin-containing 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 casein gene (Schmidhauser et al 1990, Schmidhauser et al 1992) by β 1-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 α s1-casein (Jolivet et al 2005), albumin (Liu et al 1991) and TGF- β , which is regulated negatively (Streuli et al 1993). Given that the microenvironment is comprised of a multitude of ECM molecules, we can imagine that the family of ECM-REs will be refined in the future to include “laminin-response element”, “collagen-response element”, etc, and various combinations thereof. ECM also regulates the expression of tissue-specific transcription factors, such as mammary gland factor (MGF/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).

The 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 re-expression of connexins can revert the tumor phenotype, highlights the importance of cell-cell communication in determining and responding to tissue architecture (Carystinos et al 2001). Indeed, disrupting tight junctions prevents establishment of tissue polarity and disrupts the structure of already polarized cells, leading to neoplastic growth (Itoh & Bissell 2003).

Aside from inducing signal transduction through integrins and determining tissue morphology, the microenvironment also affects the structure of the nucleus. Laminin induces histone deacetylation (Pujuguet et al 2001), and the expression of the β -casein gene can be modulated by altering the organization of histones (Myers et al 1998). Histone acetylation promotes chromosome decondensation and unfolding, increasing the accessibility to transcription factors and other regulatory machinery, thereby enhancing transcription (Jenuwein & Allis 2001). Recent experiments have demonstrated that cell rounding itself, independently of cell-ECM interactions, leads to histone modifications (J. Le Beyec, R. Xu, S.Y. Moonlee, C.M. Nelson & M.J. Bissell, unpublished data). Because the cytoskeleton appears to physically connect cell-ECM adhesions to the nucleus (Maniotis et al 1997), it is tempting to speculate that the effects due to changes in cell morphology are transmitted through the cytoskeleton. Taken together, these data

lead to an updated view of dynamic reciprocity (Figure 1B), whereby tissue specificity is determined and maintained by integrin- and non-integrin-mediated interactions 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 in the promoter regions 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 tissue-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”. Pancreas, lung, kidney, prostate, salivary and mammary gland all develop by branching morphogenesis, driven by epithelial/mesenchymal interactions, involving stimulatory signaling 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 in Section II. Interestingly, the epithelium in these organs is initially derived from different germ layers: endoderm in pancreas and lung; mesoderm in kidney; ectoderm in mammary gland. However, there are also major differences in the contexts under which each of these organs develops which likely play 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 acquisition evolutionarily (Ofstedal 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 million years ago) suggests that some of the above-described mechanisms for directing tissue specificity might be conserved [last reviewed in (Ashkenas et al 1996)]. Indeed, homologs of ECM proteins and integrins are present in many invertebrates. The nematode worm *C. elegans* expresses collagens and a β 1-integrin homolog, β_{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 *D. 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 fruit fly epithelial cells (Deng et al 2003). Hydra express laminins, collagens, MMPs, and a putative β 1-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 *D. 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). Since cytoskeleton is, in general, conserved through different phyla (Muller et al 2005), it is likely that tissue context plays an analogous role in the development, differentiation, and homeostasis of many organisms.

V. INTEGRATION

A fundamental property of all known (and, presumably, therefore successful) forms of life is the ability to adapt to changes in the environment, 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 lifetime of the organism. 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). An old breast is not the same as a young breast. As menopause approaches, epithelial cells die off, the stromal compartment alters, the entire morphology of the organ changes. It behooves us, then, to combine our knowledge of tissue specificity and developmental biology so as to generate an integrated way of thinking about development, homeostasis, cancer, and aging.

What we have argued 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 “software” 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: precisely why

development is so miraculously robust. The process of development is stochastic, not written in stone.

VI. 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.

VII. SUMMARY POINTS

- Development, differentiation, and homeostasis are controlled by a cell’s interactions with its neighbors, ECM and its receptors, ECM degrading enzymes, and soluble cues (hormones, cytokines, and growth factors);
- Physiological function requires form;
- Phenotype can be dominant over genotype.

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Annotated references

- (Bhowmick et al 2004) – Demonstrates clearly the role of the stroma in cancer development.
- (Kirschner et al 2000) – A superb synthesis of the organizing principles of biology.
- (Paszek et al 2005) – Demonstrates role of mechanical stress in control of phenotype.
- (Sakakura et al 1976) – Elegantly delineates the different roles of mesenchyme and epithelium in morphogenesis and differentiation.
- (Wessells 1977) – An excellent, concise review of the early material, especially on mesoderm induction experiments.

Acknowledgements

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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) An updated view of dynamic reciprocity.

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. In the case of mammary gland (MG) epithelium recombined with salivary gland (SG) mesenchyme, although the epithelial tree resembles salivary gland, it can still produce milk. (B) adapted from (Parmar & Cunha 2004).

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 (red) 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 (LEPs) surrounded by myoepithelial cells (MEPs) 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.

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	cMet	Amphiregulin	EGFR (ErbB1)
	IGF-I	IGF-I receptor	TGF- β	TGF β R-I, -II
	Activin/inhibin B	Activin receptors	PTHrP	PTHrP receptor
	Epimorphin	<i>unknown</i>		
	MMP-2, -3, -9, -11	n/a		
During alveolar development/pregnancy	Neuregulin	ErbB3/ErbB4		
	Activin/inhibin B	Activin receptors		
	KGF (FGF-7)	FGFR2-IIIb		
	Epimorphin	<i>unknown</i>		
	MMP-3	n/a		

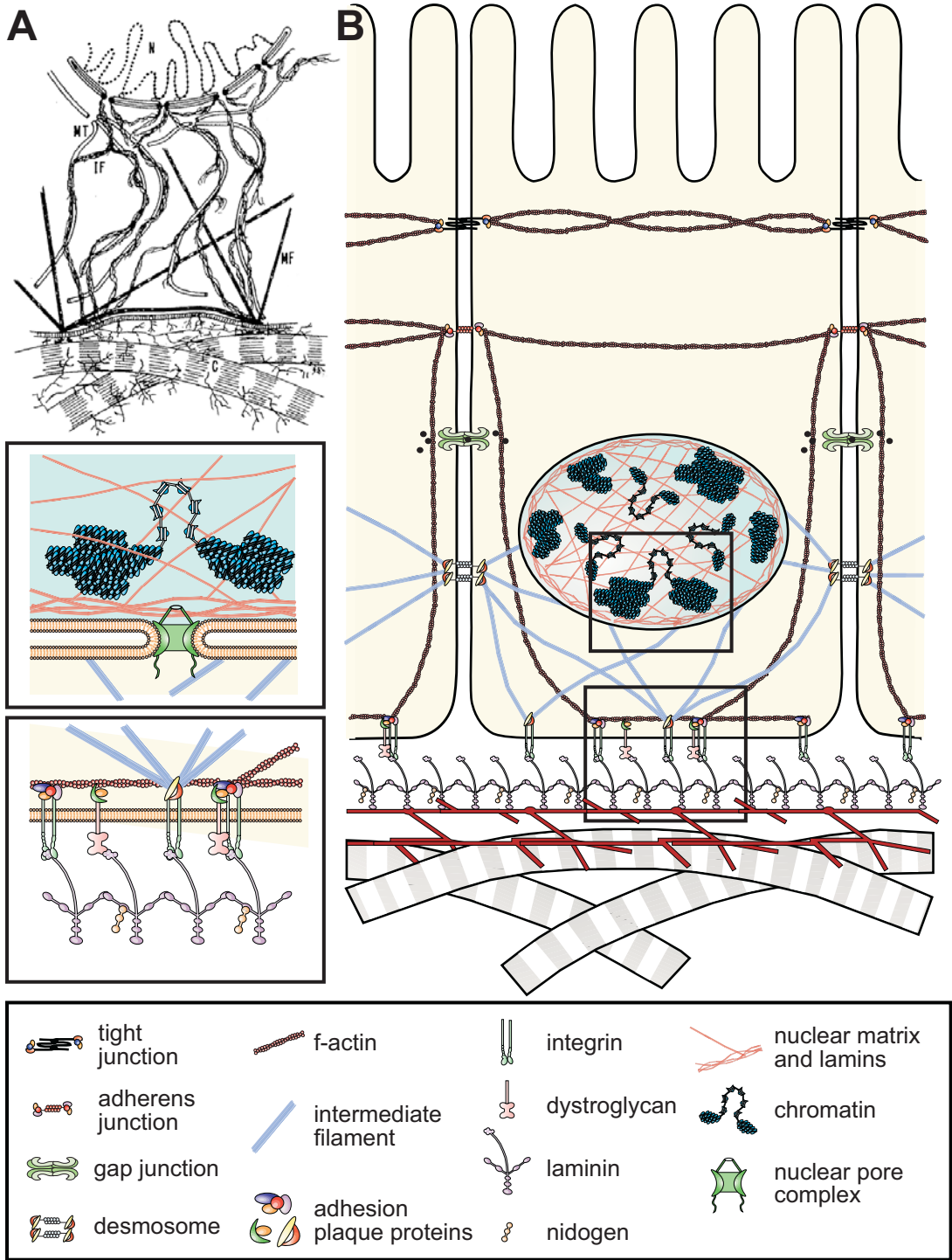


Figure 1

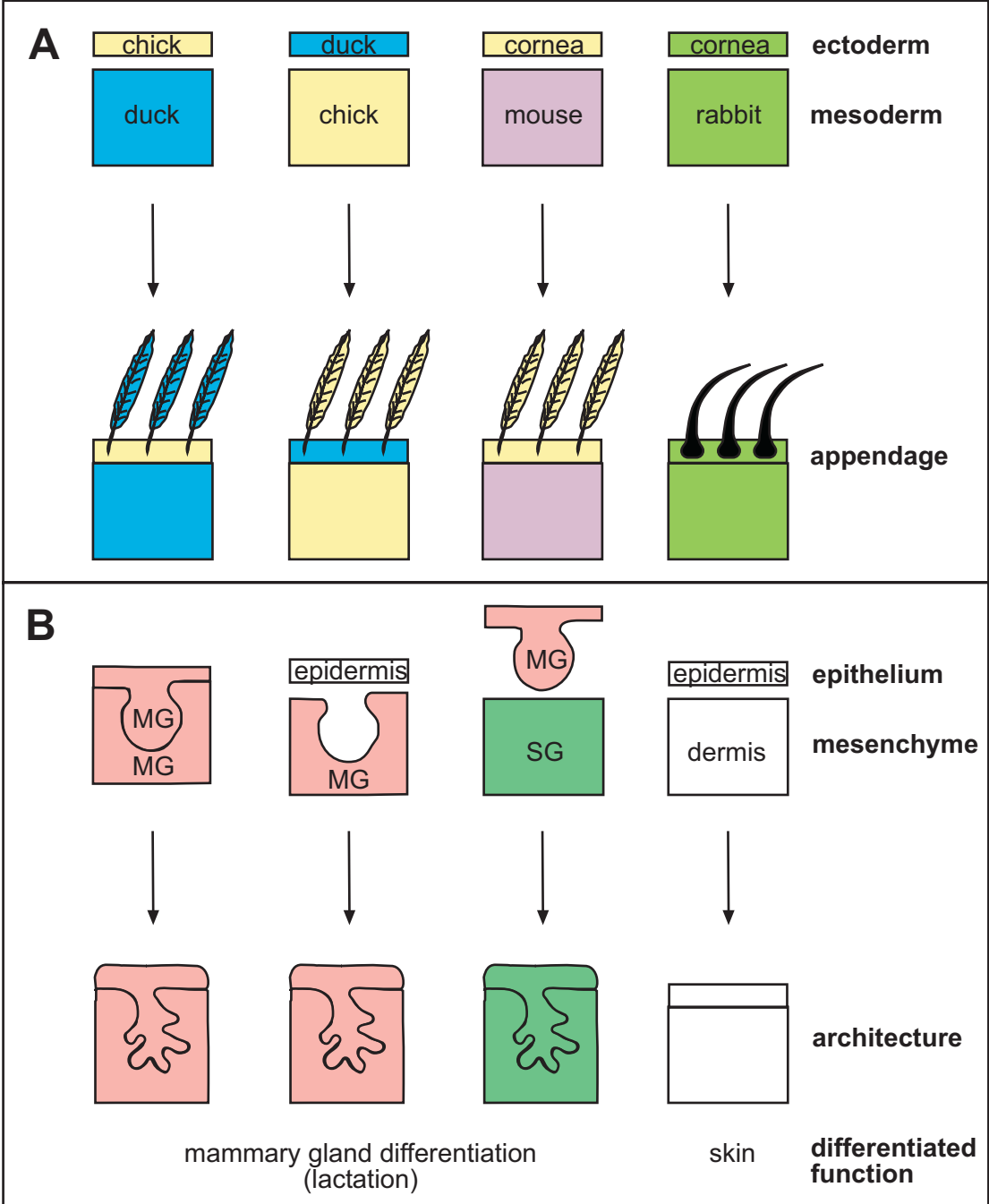


Figure 2

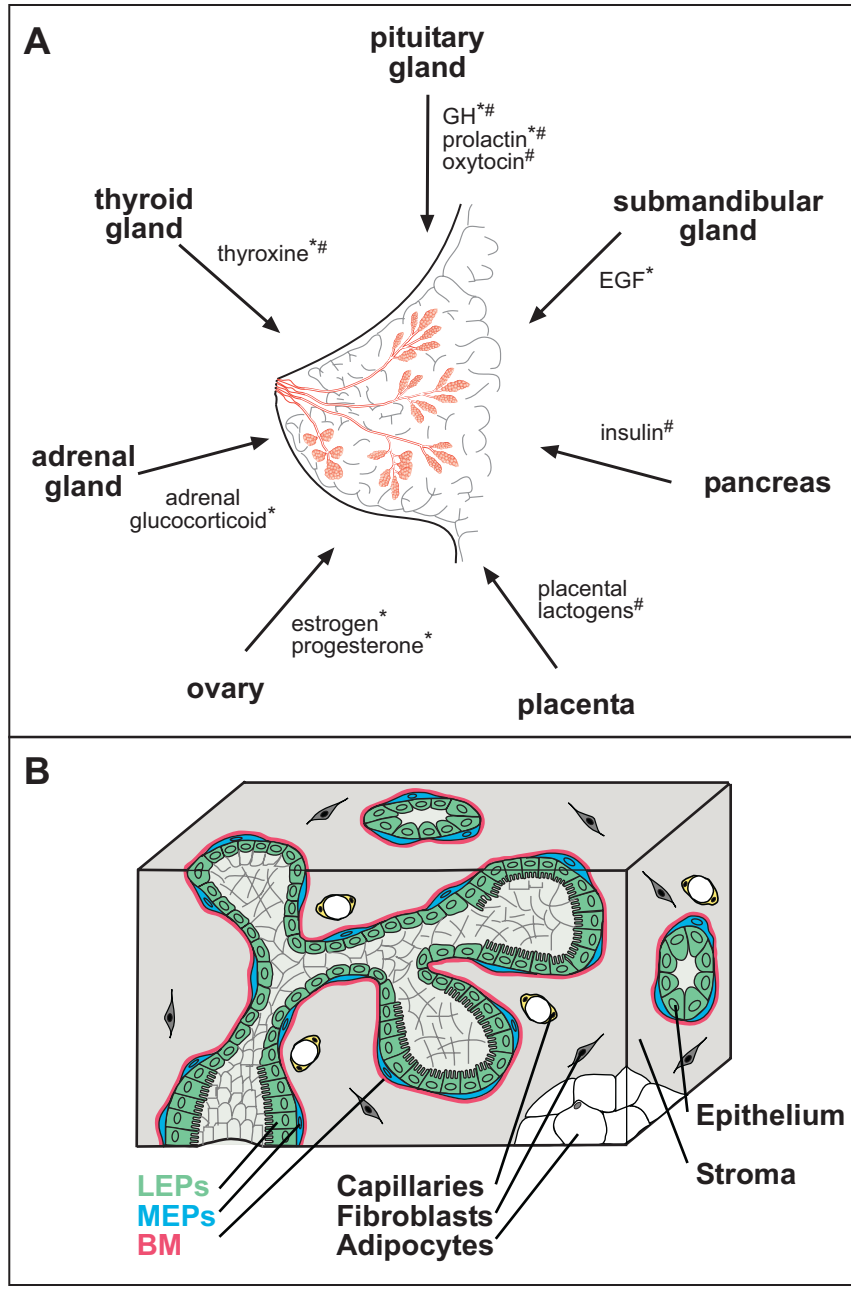


Figure 3