Dynamic Interactions Between Cells and Their Extracellular Matrix Mediate Embryonic Development

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SUMMARY

Cells and their surrounding extracellular matrix microenvironment interact throughout all stages of life. Understanding the continuously changing scope of cell-matrix interactions in vivo is crucial to garner insights into both congenital birth defects and disease progression. A current challenge in the field of developmental biology is to adapt in vitro tools and rapidly evolving imaging technology to study cell-matrix interactions in a complex 4-D environment. In this review, we highlight the dynamic modulation of cell-matrix interactions during development. We propose that individual cell-matrix adhesion proteins are best considered as complex proteins that can play multiple, often seemingly contradictory roles, depending upon the context of the microenvironment. In addition, cell-matrix proteins can also exert different short versus long term effects. It is thus important to consider cell behavior in light of the microenvironment because of the constant and dynamic reciprocal interactions occurring between them. Finally, we suggest that analysis of cell-matrix interactions at multiple levels (molecules, cells, tissues) in vivo is critical for an integrated understanding because different information can be acquired from all size scales.

INTRODUCTION

The extracellular matrix (ECM) was once thought to be merely a scaffold that occupied the spaces between cells in multicellular organisms. It was thought that ECM was only required for cell proliferation and inhibition of programmed cell death. However, the ECM is now recognized as a complex and dynamic structure that is critical for normal development and physiology. Cells interact constantly with the ECM: they adhere to/disengage from the ECM and secrete proteins that modify the ECM environment. Complex and changing cell-ECM interactions mediate cell survival, apoptosis, division, differentiation, and are a hallmark of embryonic development.

Although all tissues contain ECM, the composition of the ECM in different tissues varies tremendously. The ECM provides physical support to an organism and fills the space between cells and tissues. Not only does the ECM provide a three-dimensional substructure for cell adhesion and movement, but the ECM also acts as a storage compartment for signaling molecules. Broadly, the ECM can be thought of as a hydrated meshwork comprised of proteins with different biophysical functions. The relative proportion of proteins with different physical characteristics generates different ECMs with varying biomechanical properties. The ECM in skin is fairly elastic, the ECM in bone is very stiff, and ECM stiffness/elasticity varies in connective tissues like tendons. Thus, there is an exquisite tissue specificity to ECM components that reflects the biophysical requirements of each tissue—the epitome of coordination between form and function.

Abbreviations: CMAC = cell matrix adhesion complex; ECM = extracellular matrix; FA = focal adhesion; FB = fibrillar adhesions; FC = focal complex; FGF(R) = fibroblast growth factor (receptor); Fn = fibronectin; GAG = glycosaminoglycan; MMP = matrix metalloprotease; PCP = planar cell polarity

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Why is regulation of the interactions between cells and the ECM critical? This can perhaps best be understood with an imperfect (but informative) analogy. Imagine a cell as a hiker who can run up a well maintained gentle slope, painstakingly scramble up a steep slope, don crampons and rope up with other hikers to climb Mt. Rainier, or anything in between. In the case of the hiker, many choices are made: where to start, where to finish, how to get there, what footwear and clothing is most appropriate for the conditions, etc. The initial decision to hike up an easy climb in good weather can turn disastrous if conditions change and the hiker must adjust their path and/or clothing and gear.

Although migrating cells do not “choose” their path and instead follow their genetic program, their behavior is complex and modified by constant interactions with the ECM. Cells can migrate individually or in groups. The speed and directionality of migration are critically dependent upon the extracellular environment. Migration tends to be slower in highly adhesive environments and faster in less adhesive environments. Cells can migrate on different matrices (the mountains) and with different receptors (the clothing and footwear of the hiker). The interaction of cells with their extracellular environment is dynamic like the conditions on a mountain and cells can adapt to perturbations, that is, a change in the weather.

ECM composition within the same tissue is also dynamic and reflects the delicate balance between ECM degradation and synthesis. The composition of the ECM within a tissue changes not only during morphogenetic events in early development, but also in aging and disease. One large focus of current research is to understand how the normal balance between ECM degradation and synthesis is maintained, with the goal being to understand how this system is deregulated during aging and disease.

The vast majority of studies investigating cellular adhesion to the ECM have been done in vitro. It is clear from these studies that regulation of cell–ECM adhesion occurs on many different levels including gene expression, alternative splicing, translational regulation, post-translational modification, and formation of multiprotein complexes. The next critical step for developmental biologists is to understand cell behavior in vivo. Pioneering efforts from a number of laboratories generated targeted mouse knockouts and showed that: (1) inhibition of cell–ECM adhesion is frequently lethal and (2) different components of the cell–ECM adhesion machinery play specific roles during embryonic development (Aszodi et al., 2006). These experiments showed that cell–ECM adhesion plays a critical role during embryonic development. However, due to early embryonic lethality, they did not elucidate many of the underlying mechanisms driving cell–ECM interactions.

The current challenge is to use new advances in technology to study the dynamics of cell–ECM adhesion in vivo. In particular, understanding the intimate, bi-directional relationship between cells and their local ECM environment is critical. ECM remodeling accompanies cellular shape changes, cell migration, and differentiation. ECM remodeling can also influence cell signaling via the release of secreted signaling molecules previously sequestered within the ECM. ECM remodeling is induced by many different signals, including cellular tension and growth factor signaling. Thus, remodeling of the ECM allows cells to integrate signaling and differentiation with the structure of their local microenvironment. The term “dynamic reciprocity” was introduced to promote the view that tissue architecture is critically dependent upon reciprocal signals between cells and their extracellular environment (Bissell et al., 1982). The intent of this review is to provide the reader with a broad overview of: (1) the myriad of ways in which cell adhesion to the ECM is regulated and (2) how this regulation is critical for development and physiology. All of the proteins mentioned herein are themselves the primary subjects of multiple reviews. This review focuses more on studies that integrate dynamic interactions between cells and their ECM.

It is worth noting that one current trend in biology is the undertaking of “omics-type” studies. This large scale, high-throughput approach is critical and invaluable. However, one of the concepts that we would like to convey, through examples illustrated below, is that detailed analysis of cell–ECM interactions is also absolutely fundamental to understanding embryonic development as well as the transition to disease states. It is our opinion that a comprehensive picture of cell behavior will emerge only by combining the information learned from studies at all size scales, along with new imaging technologies and modeling efforts.

COMPONENTS INVOLVED IN CELL–ECM ADHESION

Cell–ECM adhesion involves: (1) ECM proteins themselves, (2) transmembrane receptors that bind ECM ligands (integrins are the major class of transmembrane ECM receptors), and (3) intracellular proteins that directly or indirectly bind to integrins and/or the cytoskeleton to affect/downstream signaling. We will discuss some of the most well-known proteins in each class.

Extracellular Matrix Proteins

Although the major structural components of different ECMs are the same, their relative proportions and types vary, resulting in strikingly different physical properties. One unifying feature of different ECMs with diverse functions is the ability to maintain shape and to flexibly respond to deformation. The major structural components of the ECM include: collagens, proteoglycans, noncollagenous glycoproteins, and elastins (Fig. 1). These components play complementary biophysical roles. Maintenance of structural integrity is predominantly via collagens, which are the most abundant protein in the human body. Collagen is a major component of connective tissues such as skin and cartilage. These tissues flexibly maintain shape by absorbing deformation. Clearly, however, skin and cartilage have different biophysical properties. There are different collagen types (fibrillar, globular) that generate varying three-dimensional scaffolds. In addition, the alignment and density of collagen are the primary determinants of the tensile strength of the ECM (Kjaer,
The importance of collagen is highlighted by the fact that mutations in collagens type I, III, V, or type III procollagen can result in Ehlers-Danlos syndrome, which is characterized by skin laxity and joint hypermobility (Superti-Furga et al., 1989; Sillence et al., 1991; Nicholls et al., 1996; Toriello et al., 1996; De Paepe et al., 1997; Smith et al., 1997). Synthesized collagen scaffolds show great promise for tissue engineering and regeneration. Some current efforts directed towards understanding collagen elasticity involve applying engineering concepts to molecular mechanisms (Scott, 2003; Chaudhry et al., 2009). The potential for engineered collagen scaffolds to be used as transient and bioresorbable cartilage, skin, and cardiovascular grafts is exciting and is the focus of much research.

Proteoglycans are a protein plus a glycosaminoglycan (GAG). The GAG portion of proteoglycans has a net negative charge that attracts water (and cations) to form a hydrous gel that resists compressive forces. Proteoglycans regulate collagen fibrillogenesis. Thus, proteoglycans can indirectly regulate tissue remodeling in response to mechanical forces by modifying the amount, type, and alignment of collagen fibrils. The application of tensional or compressive loads to excised bovine tendons changed the amount and composition of proteoglycans (Robbins et al., 1997). Proteoglycan composition and amount can also change in vivo. An increase in proteoglycans and GAG content was observed in tendons from 6.5 weeks old chickens that had been exercised on treadmills compared to their more sedentary counterparts (Yoon and Halper, 2005). In addition to modulating the physical properties of the ECM in response to mechanical stress, proteoglycans also modulate the movement and activity of signaling molecules through the ECM. Perlecan is an exceedingly large, ubiquitous ECM protein that surrounds cells. It has been proposed that Perlecan acts as a clearing house that controls local levels of mitogens and morphogens (Whitelock et al., 2008). The complex phenotype of mice mutant for Perlecan attests to the importance of proteoglycans for normal development (Arikawa-Hirasawa et al., 1999; Costell et al., 1999).

Glycoproteins are proteins with sugar modifications. Two prominent noncollagenous glycoproteins, fibronectin (Fn) and laminin, are discussed in further detail below. Finally, elastins, as their name suggests, are remarkable proteins that are resistant to shape deformation and are primarily responsible for the elasticity of connective tissues (Debelle and Tamburro, 1999; Tatham and Shewry, 2000; Li and Daggett, 2002; Mithieux and Weiss, 2005; Daamen et al., 2007). Elastin-based biomaterials are becoming popular for tissue engineering due to their elastic properties, the stable nature of insoluble elastin (half-life of 70 years), and the
potential for self-assembly. Perhaps the most famous bioelastic material is flagelliform spider silks, which can extend approximately 200% of their length without breaking (Xu and Lewis, 1990; Dong et al., 1991; Gosline et al., 1999)!

Spotlight on fibronectin. Fibronectin is a major component of many extracellular matrices and plays roles in both development and disease. Fn is perhaps most renowned for its functions during branching morphogenesis and cell migration (Roman et al., 1991; Roman, 1997; Jiang et al., 2000; Sakai et al., 2003; Trinh and Stainier, 2004; Davidson et al., 2006; Larsen et al., 2006b; Matsui et al., 2007). Fn provides an excellent example of the regulation of cell–ECM adhesion components at multiple levels. Fn transcription during embryonic development is spatially and temporally regulated (Julich et al., 2005; Koshida et al., 2005; Matsui et al., 2007). Post-transcriptionally, there are many alternatively spliced forms of Fn. The expression of different splice variants frequently correlates with disease and is regulated by multiple pathways (White et al., 2008). Fn regulation is further complicated by the fact that there are two forms of Fn: (1) plasma Fn (pFn) which circulates in the blood and is globular and (2) cellular Fn (cFn) which is assembled into fibrils that in turn polymerize to form a fibrillar matrix. Fn fibril formation is regulated on many levels, including dimerization and integrin binding, as well as stretch-mediated uncovering of a cryptic self-binding site (Leiss et al., 2008). The ability of fibrillar Fn to be stretched up to fourfold by living cells may be particularly relevant for dynamic cell movements that occur during development (Erickson, 2002).

One critical function of Fn is to regulate deposition of collagens type I and III in the ECM (Sottile and Hocking, 2002; Velling et al., 2002). Interestingly, it has recently been shown that the ability of Fn to bind to collagen is critical for Fn-stimulated cell migration during wound healing (Sottile et al., 2007). Although this study was in vitro, the dynamic interaction between Fn-dependent collagen deposition and Fn-dependent cell migration highlights the need for integration of multiple ECM components when studying developmental processes.

In the past decade, Fn has also been shown to be a critical regulator of branching morphogenesis. Branching occurs most frequently in epithelial tissues and involves repeated elongation and forking to generate the complex shapes of lungs, kidneys, and mammary glands, for example. As branching bears some similarities to fractals, mathematicians and biologists have long been fascinated by the morphogenetic process. An excellent and entertaining book devoted to branched systems begins with Leonardo da Vinci’s early studies of tree dimensions, and is highly recommended for interested readers (Davies, 2005). Here, we will focus on recent results highlighting dynamic roles for Fn during branching morphogenesis. One model for branching morphogenesis is the mouse mandibular salivary gland. Branching occurs first by the formation of localized, shallow clefts, which then deepen to become new buds (Grobstein, 1953). A key study showed that Fn expression transiently increases at cleft epithelial cells just as narrow clefts form. The increase in Fn expression correlates with a decrease in the cell–cell adhesion protein, E-cadherin (Sakai et al., 2003). Fn expression decreases as clefts broaden. Incubation of salivary glands with an antibody against Fn inhibited branching whereas adding exogenous Fn increased branching (Sakai et al., 2003) (Fig. 2A). Thus, the authors proposed that localized Fn assembly at clefts results in the conversion of E-cadherin-based cell–cell adhesion to integrin-based cell–ECM adhesion, resulting in cleft progression. This pioneering experiment not only elucidated a key role for Fn in branching morphogenesis, but also highlights the dynamic role of ECM regulation during morphogenesis.

One key question that arose, however, was how adding exogenous Fn could promote branching if Fn was providing a local cue for cleft formation. A very elegant study addressed this issue by asking if directional Fn assembly might provide the impetus for cleft formation. Mouse mandibular salivary glands were incubated in Alexa-fluor conjugated Fn, which was then washed out and replaced by unlabeled Fn (Larsen et al., 2006b). This experiment showed that older Fn remains coherent, and is actually translocated inward as newer Fn is assembled behind it. Thus, the polarity of Fn assembly may provide a critical local cue for cleft progression (Larsen et al., 2006b) (Fig. 2B). The “wedge” of Fn would also result in conversion of cell–cell adhesion to cell–matrix adhesion (Larsen et al., 2006b). One critical question that remains to be addressed is whether Fn plays a signaling role in this adhesion conversion, or the progression of the Fn wedge mechanically breaks cell–cell adhesion. These studies, however, illustrate recurring themes of ECM regulation: ECM is dynamic, can play signaling and mechanical roles, and ECM modulation interacts with cell–cell adhesion as well as cell–ECM adhesion.

Spotlight on laminins. Laminin is necessary, along with collagen type IV, for the assembly of the basement membrane, a specialized part of ECM that provides a physical structure delineating cells from the ECM and also modulates cell signaling (Engvall, 1995; Gullberg et al., 1999; Miner and Yurchenco, 2004; Aumailley et al., 2005; Nguyen and Senior, 2006; LeBlu et al., 2007; Tzu and Marinkovich, 2008). Laminins are cross-shaped heterotrimeric proteins comprised of an α, β, and γ chain. Five α, four β, and three γ chains (Miner and Yurchenco, 2004) assemble in different combinations to generate the 16 known isoforms (Aumailley et al., 2005) whose names reflect their composition (e.g., laminin 332, which is α3β3γ2). Assembled laminin proteins self-polymerize, along with collagen type IV, to form basement membranes. Tissue specific function of laminin isoforms during development arises not only through tissue specific expression of different laminin chains, but also through the generation of splice variants and post-translational glycosylation and processing. An excellent recent review (Tzu and Marinkovich, 2008) highlights the intricacies of laminin structure, the relationship between structure and function, and also includes a comprehensive list of knockout mice and their phenotypes.

Recent studies have provided new insight into roles for laminin during boundary formation. The formation of ECM-rich boundaries is an absolutely fundamental aspect of development. Boundary formation can separate and
compartmentalize initially homologous cells. This compartmentalization occurs in the hindbrain during rhombomere formation and in the paraxial mesoderm during somite formation. Boundaries can also divide different tissues. For example, the notochord–somite boundary separates notochord cells from the paraxial mesoderm. In addition to separating structures, boundary formation contributes significantly to generating the three-dimensional shape and structure of developing organisms. The cell biology of boundary formation has been elucidated in many contexts. During Xenopus development, the notochord–somite boundary functions to “capture” or “trap” intercalating notochord cells. In this instance, intercalating notochord cells exhibit protrusive activity as they elongate. Upon reaching the notochord–somite boundary, protrusive activity and elongation cease. This has been termed “boundary capture” (Keller et al., 2000). However, the ECM proteins responsible for boundary capture are not known. One hint that laminin might play a role in the formation of the notochord–somite boundary came from analysis of zebrafish mutations in two laminin chains, laminin β1 and γ1. In these embryos, notochords form but are not maintained (Parsons et al., 2002). Within the last year, two papers have shed new light on roles for laminin in boundary formation. In ascidians, laminin plays a role in “trapping” or “boundary capture” of intercalating notochord cells (Veeman et al., 2008). In the absence of laminin, notochord cells invade and can cross the notochord–somite somite boundary. In the context of myotome boundary formation in zebrafish, laminin is required to trap elongating muscle cells. It was not known whether laminin in the basement membrane ceases elongation of muscle cells simply by providing a physical barrier, or by signaling prompted by laminin binding ceases elongation. Genetic mosaic analysis suggested that laminin signaling within the myotome boundary plays a role in trapping elongating muscle cells (Snow et al., 2008a). Future work will likely focus on the signaling mechanisms that lie downstream of “boundary capture.”

Metalloproteases. Much of the dynamic remodeling of the ECM is dependent upon matrix metalloproteases (MMPs) and members of the a disintegrin and metalloprotease (ADAM) and a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) families. Pro-MMPs lie dormant within the ECM and are activated by various signals (Murphy et al., 1999; Davidson et al., 2002; Tsuruda et al., 2004; Nagase et al., 2006; Amalinei et al., 2007; Ra and Parks, 2007; Clark et al., 2008; Filiz et al., 2008; Fu et al.,
2008). Misregulation of MMP activity contributes to multiple diseases (metastatic carcinoma, cardiovascular disease, rheumatoid arthritis, osteoarthritis, oral diseases) and is also implicated in preterm labor (Davidson et al., 2002; Abraham et al., 2005; Hofmann et al., 2005; Vadillo-Ortega and Estrada-Gutierrez, 2006; Lemaître and D’Arminio, 2006; Liu et al., 2006; Mon et al., 2006; Cockle et al., 2007). The dynamic interaction between ECM synthesis/assembly and degradation via MMPs is an exquisite balancing act that mediates both embryonic development and tissue homeostasis. For example, a migrating cell must release sufficient proteases such that it can move, but not too many such that excessive matrix degradation leads to loss of cellular traction forces. The mechanisms that underlie the regulation of this balancing act are currently the subject of intense research with the hopes that it can be exploited for therapeutic purposes.

Transmembrane Receptors for ECM Proteins

Integrins are a major class of ECM receptors that transmit signals both from the ECM to within the cell (“outside-in” signaling) and from within the cell to the ECM (“inside-out” signaling) (Arnaout et al., 2005). Binding of integrins to the ECM results in the assembly of complexes that link to and coordinate cytoskeletal morphology. Thus, integrins and their associated proteins physically link the ECM to the intracellular cytoskeleton. As integrins do not have inherent enzymatic activity, many of the proteins involved in complex assembly are kinases or phosphotases. Integrin ligation can also result in changes in the molecular composition of the ECM. Integrins are transmembrane heterodimers comprised of an α and a β subunit. Both α and β subunits have large extracellular domains and relatively short cytoplasmic domains (Arnaout et al., 2005). Twenty-four heterodimers formed from the 18 known α and 8 known β subunits have been identified in humans (Takada et al., 2007). Although integrin receptors are notoriously promiscuous, they are receptors for α-subunit of a large number of ligands. For example, integrin α5β3 is a receptor for Fn, osteopontin, fibrillin, L1, thrombospondin, and ADAM family members. Conversely, integrin α5 is one of six known receptors for Fn (Humphries et al., 2006). Integrin expression during development is regulated both spatially and temporally, and multiple analyses indicate discrete roles for different integrins during vertebrate development (Sheppard, 2000; Bokel and Brown, 2002; Julich et al., 2005).

Integrins are highly responsive receptors that can exist in a bent (inactive) or an extended (active) state (Humphries et al., 2004). Post-translational modification of integrins can alter their affinity for ECM components and thus the overall adhesiveness of cells. One such example is the ADP-ribosylation of integrin α7 that occurs during myotube development in vitro. Integrin α7 is ADP-ribosylated at multiple sites, depending upon NAD concentration. This ADP-ribosylation by an ADP-ribosyltransferase is not readily reversible (Zolkiewska and Moss, 1995) and leads to increased laminin-binding affinity of integrin α7β1 (Zhao et al., 2005). Given that NAD is an obligatory substrate in ADP-ribosylation reactions, the authors hypothesized that integrin α7 ADP-ribosylation by this ecto-ADP-ribosyltransferase might be a protective mechanism that increases integrin α7β1 adhesion to laminin when muscle cell membranes have been compromised (as frequently occurs in muscular dystrophies) and intracellular NAD leaks into the extracellular space (Zhao et al., 2005). Although this paradigm has not yet been investigated in vivo, it is an excellent example of how post-translational modifications of integrins can affect their function.

Key Intracellular Components

Given the diverse functions of cell–ECM adhesion and the rapid assembly and disassembly of adhesion complexes, it is perhaps not surprising that different types of complexes that anchor the cell to the ECM can form. These differing classes have been defined with in vitro studies. The parameters that define the classes include the size, constituency, and signaling effects. The context in which a cell is located—the differentiation state of the cell, the specific ECM to which a cell is adhering, the physical forces of the tissue—impacts the type and size of complexes formed (Romer et al., 2006). The classes, from smaller to larger, are FCs (focal complexes), FAs (focal adhesions), and FBs (fibrillar adhesions) (Geiger et al., 2001). A recent review, noting the controversy over the definitions, suggests an overarching term for these complexes: cell matrix adhesion complexes, or CAMCs (pronounced see-macs) (Lock et al., 2008). This review is an excellent commentary on the dynamics of cellular adhesion, and suggests a systems biology approach to studying CAMCs.

CAMCs contain dozens, if not hundreds, of proteins. Most of the proteins in CAMCs are post-translationally regulated by phosphorylation and/or glycosylation, for example (Fig. 3). This regulation adds yet another layer of dynamic flexibility that mediates a cell’s response to ECM adhesion. Some key components of CAMCs (integrin-linked kinase, kindlins, paxillin, talin, zyxin) are the sole subjects of several recent reviews (Beckerle, 1997; Wang and Gilmore, 2003; McLean et al., 2005; Mitra et al., 2005; Boulter and Van Obberghen-Schilling, 2006; Legate et al., 2006; Hannigan et al., 2007; Deakin and Turner, 2008; Larjava et al., 2008; McDonald et al., 2008; Chatzizacharias et al., 2008a,b) and thus will not be discussed here in detail.

Spotlight on focal adhesion kinase (FAK) and muscle differentiation. Attachment of muscle fibers to laminin in the basement membrane is necessary for musculoskeletal function. Mutations in genes that inactivate proteins required for adhesion to the basement membrane lead to multiple myopathies such as Duchenne, Becker, merosin-deficient, and many of the limb girdle muscular dystrophies (Hoffman et al., 1987; Tome et al., 1994; Bonnemann et al., 1995; Mayer et al., 1997; Hayashi et al., 1998). Many lines of evidence suggest that one therapeutic option for treatment of myopathies is to restore adhesion of myotubes (bundles of muscle fibrils) to the basement membrane. These studies indicate that the exact mechanism of increased adhesion is not critical. Thus, multiple studies have focused on elucidat-
appears to be involved in the formation of strong, long-lasting CMACs that mediate muscle-tendon adhesion. FAK activation, or autophosphorylation on pY397, can also be induced by mechanical stress in cardiac myocytes (Fonseca et al., 2005). The activation of FAK correlates with many events during myotube formation in cell culture. FAK is activated via a PKC dependent pathway during insulin-mediated skeletal muscle cell spreading (Goel and Dey, 2002a). FAK expression and activation are modulated in response to mechanical force in vivo, and the response is different in predominantly slow-twitch versus predominantly fast-twitch muscles (Fluck et al., 1999; Gordon et al., 2001). During the differentiation of C2C12 myoblasts into myotubes, the ratio of inactivated FAK to activated FAK is transiently decreased, but then increases and activated FAK localizes to the tip of actin stress fibers. These data indicate roles for FAK in myotube morphogenesis in vitro and in vivo. FAK function in muscle development and the signaling that occurs through CMACs is still under investigation.

**BROAD FUNCTIONS OF CELL–ECM ADHESION**

**Cell–ECM Interactions Regulate Cell Signaling, Tissue Integrity, and Homeostasis**

The ECM within all tissues provides a necessary scaffold for generation and maintenance of tissue architecture. It is impossible to list the plethora of functions that the ECM plays in development and physiology because virtually every developmental and physiological process relies, either directly or indirectly, on cell–ECM interactions. Cell–ECM interactions also modulate cellular responses to major signaling pathways. One major signaling pathway includes the fibroblast growth factors (FGFs) and their receptors (FGFRs). Recent experiments elucidating the extracellular "interactome" of the FGF receptor–ligand system have shown that ECM receptors act as noncanonical co-receptors that impart specificity to FGF signaling (Polanska et al., 2008). For example, inhibition of alpha V integrin can block FGF induced cell adhesion, migration, and angiogenesis (Rusnati et al., 1997; Santulli et al., 2008). This type of regulation provides mechanistic insight into how crosstalk between cell adhesion receptors and signaling pathways mediates cellular behavior.

Another mechanism for ECM-mediated regulation of growth factor signaling is the extensive crosstalk observed between growth factor and integrin signaling pathways. Integrin-based adhesion to the ECM is required for some cells to respond to growth factor signaling (Giancotti, 1996, 1997, 2000, 2003; Frisch and Ruoslahti, 1997). For example, the proliferation and/or differentiation of many cell types during development requires both adhesion to the ECM as
well as the appropriate growth factors (Sastry and Horwitz, 1996). Since the first experiments demonstrating crosstalk between growth factor signaling and integrin mediated signaling, it has become clear that crosstalk is frequent, dynamic, and involves a complex network of interactions (Schwartz and Ginsberg, 2002; Comoglio et al., 2003; Chan et al., 2006; Larsen et al., 2006a; Muller et al., 2008). Many laboratories are endeavoring to elucidate and exploit the complexity of integrin-growth factor signaling crosstalk with the hopes that more specific therapeutics with fewer side effects can be developed.

Adhesion to the ECM modulates expression and activation of growth factors and growth factor signaling can, in turn, regulate cell adhesion. One recently studied example is the pleiotropic cytokine transforming growth factor beta 1 (TGF-β1) that is sequestered as a large latent protein complex within the ECM. Misregulated activation of this complex results in excess TGF-β1 signaling that promotes myofibroblast differentiation and ultimately contributes to fibrosis in many organs. Activation of latent TGF-β1 is dependent upon tension, ECM composition, and the interaction of integrin receptors with MMPs (Wipff et al., 2007; Wipff and Hinz, 2008). This paradigm is just one example of the complex regulation of growth factor release from ECM stores.

ECM receptors are both modulated and activated by signaling pathways. Notch signaling plays a major role in regulating cell fate and differentiation during development. Angiogenesis is a complex process that involves Notch signaling. Notch-4 inhibits angiogenesis by increasing integrin β1-mediated adhesion to collagen and, thus inhibiting sprouting of endothelial cells from preexisting vessels. Interestingly, ectopic expression of activated Notch-4 does not increase the levels of integrin β1, but rather results in more integrin β1 molecules adopting the active, high affinity conformation (Leong et al., 2002). The mechanism underlying integrin activation by Notch-4 is not known. However, recent results suggest that Notch-1 mediates integrin activation via activating R-ras (Hodkinson et al., 2007). Thus, the “ECM interactome” is ubiquitous and may play a central role in integrating cellular responses to multiple signaling pathways.

Disrupted cell–ECM interactions can also perturb homeostasis. It is becoming increasingly recognized that disrupted interactions between cells and the ECM underlie the etiology of metastatic carcinomas, cardiovascular diseases, muscular dystrophies, kidney dysfunction, and skin disorders, among many other diseases and syndromes (Nelson and Bissell, 2006; Spinaile, 2007; Stenina et al., 2007; Hamilton, 2008; Libby, 2008; Tzu and Marinkovich, 2008). Many of the less comfortable aspects of aging also result from decreased strength of cell–ECM interactions. For example, tendon failure leading to tendinopathies is exacerbated by age due to load-induced degradation of ECM (Dudhia et al., 2007). Osteoarthritis occurs when abnormal cell/ECM signaling disrupts the homeostasis of cartilage and bone tissue (Iannone and Lapadula, 2003). Age-related macular degeneration can also stem from abnormal ECM (Zarbin, 2004). Clearly, proper regulation of cell–ECM adhesion is critical for physiological homeostasis and the prevention of certain diseases.

Cell–ECM Interactions Regulate Cell Migration

The extracellular milieu influences migration, signaling, proliferation, and death of cell cohorts as well as individual cells. Cell migration requires dynamic interactions of cells with their ECM: adhesion assembly and disassembly are both necessary for migration. One classic system for studying roles for cell–ECM interactions during embryonic development is the neural crest. Neural crest cells originate from the dorsal neural tube and migrate great distances to give rise to neurons, pigment cells, cartilage, and connective tissue as well as other cell types. From the first descriptive data showing expression of cell–ECM adhesion components during neural crest migration (Krotsoski et al., 1986; Krotsoski and Bronner-Fraser, 1990) to functional studies shortly thereafter (Bronner-Fraser, 1986; Lallier and Bronner-Fraser, 1991, 1992, 1993; Perris et al., 1993a,b; Lallier et al., 1994; Kil et al., 1998; Peters et al., 2002), neural crest migration has proved to be an excellent model system to study cell migration. Recent advances in understanding neural crest cell migration have shown that efficient migration requires internalization and recycling of ECM receptors. Interestingly, internalization and recycling of ECM receptors is dependent upon the composition of the ECM in vitro—an example of dynamic reciprocity that may also function in vivo (Strachan and Condic, 2004). There is exquisite specificity within this system: trunk and cranial neural crest cells respond differently to varying concentrations of Fn when integrins are activated. Trunk neural crest cells are unaffected by increasing Fn concentration, whereas cranial neural crest cells slow with increasing Fn concentration (Strachan and Condic, 2008). It will be exciting to determine if similar mechanisms function in vivo. The complexity of cell migration in vivo is apparent in that another component of the ECM, laminin 111, also regulates neural crest cell spreading, migration, and survival. These different cell behaviors are regulated by the interaction of cell surface receptors with different laminin binding domains (Desban et al., 2006). Although neural crest is by no means the only paradigm of cell migration during development, neural crest migration has been an exceedingly informative model of dynamic cell–ECM interactions regulating cell behavior.

FUNCTIONS FOR DYNAMIC MODULATION OF CELL–ECM ADHESION IN VIVO

Many groups have devised elegant and novel techniques to elucidate the molecular mechanisms of morphogenesis during development. Unfortunately, there is only space to highlight a few recent and especially intriguing studies.

Convergent Extension During *Xenopus* Gastrulation Involves Dynamic Interactions Between Cells and Their Environment

One excellent model system for the study of how polarized interactions of cells with the ECM can drive morphogenesis is convergent extension. During convergent extension, the intercalation of cells in one plane causes elongation of the tissue in the perpendicular plane. *Xenopus*
had long served as a model for the study of cell behaviors and for convergent extension in particular. In Xenopus, convergent extension of the embryonic axis during gastrulation is driven by mediolateral intercalation behavior (Shih and Keller, 1992a,b). Cells exhibiting mediolateral intercalation behavior become polarized in that they elongate and extend lamellipodia in the direction of interdigitation. Convergent extension depends upon this cell polarization and bipolar lamella formation.

Cell polarization during convergent extension during Xenopus gastrulation requires interpretation of spatial information and interactions with the ECM. Surprisingly, in this context, the planar cell polarity (PCP) signaling cascade mediates both cell polarization and interactions with the ECM. The PCP cascade activates the small GTPases rho and rac that are critical for polarized lamellipodia formation (Habas et al., 2003; Tahinci and Symes, 2003). In addition, PCP signaling modulates the ECM protein Fn in two ways: (1) to regulate normal deposition of the Fn matrix and (2) to effect cellular polarization in response to the Fn matrix (Goto et al., 2005) (Fig. 4). Thus, future studies need to consider the potential dual requirements for the PCP cascade during other morphogenetic processes (Wallingford, 2005).

At this point, the mechanisms by which Fn mediated cellular polarization were not known. Subsequent elegant experiments showed that adhesion of mesodermal cells to Fn via integrin α5β1 functions in two ways to promote mediolateral intercalation behavior: (1) Fn binding represses the number of cellular protrusions extended and (2) Fn binding promotes the mediolateral orientation of protrusions required for effective convergent extension (Davidson et al., 2006). An important insight derived from this study is that binding to Fn is required to initiate, but not maintain, mediolaterally polarized cell morphology. The authors used both chronic and acute disruption of Fn binding to separate initiation and maintenance (Davidson et al., 2006). Clearly, these results not only provide new molecular insight to the regulation of cell behavior by Fn, but should also encourage researchers to design experiments to distinguish between short and long-term requirements for cell–ECM adhesion.

One common theme of the above experiments is that the same signaling cascade or protein can play more than one role within the context of the same process. The methods used to parse multiple roles differed: one study asked if a Fn surface could rescue polarity and the other used chronic versus acute disruption of Fn-mediated adhesion. The progressive nature of morphogenesis further complicates a mechanistic understanding of embryonic development. In other words, processes that occur later in development necessarily rely on earlier morphogenetic events. It is thus sometimes difficult to attribute cause and effect. Towards this end, we have recently applied a fractal-based method that quantifies cellular structure within a lattice to show that Fn plays distinct roles through time in the same tissue (Snow et al., 2008b). Taken together, these experiments all highlight the spatial and temporal complexity of cell–ECM interactions during development, and provide different tools with which to tease apart discrete requirements for cell–ECM interactions during morphogenesis.

In Vivo Observation of Fn Dynamics and Correlation With Protrusive Activity

One critical method for elucidating the dynamics of cell interactions with the ECM is high-resolution time-lapse analysis of these interactions. Recently, live imaging of Fn and cellular protrusions in Xenopus animal cap explants has provided new insights into Fn fibril assembly (Davidson et al., 2008). Interestingly, there was not a strong correlation between lamellipodia extension and Fn fibril movement, and Fn fibrils move without apparent contact with lamellipodia. It was shown that there are two mechanisms that underlie fibril thickening and that they occur on different time-scales. Fn polymerization can take hours, but fibrils can also shorten...
and thicken within minutes. Not surprisingly, disruption of the actin cytoskeleton resulted in fibril collapse. Taken together, these novel experiments show that it is possible to visualize cell–ECM dynamics within a tissue and show that different ECM remodeling events can occur on different time-scales (Davidson et al., 2008). The lack of clear correlation between Fn fibril dynamics and cellular protrusions is somewhat confusing given the data mentioned above showing clear roles for Fn binding in regulating cell protrusions. However, different populations of cells were observed and it would be very interesting to use these newly developed tools to further study the acquisition and maintenance of mediolateral intercalation behavior. Furthermore, the use of GFP-tagged integrins in live embryos (Julich et al., 2005; DeSimone et al., 2007) concurrent with visualization of the ECM should add further insights.

But Do Cells Actually Migrate Across Fn?

“Galileo described the concept of motion relativity—motion with respect to a reference frame—in 1632. He noted that a person below deck would be unable to discern whether the boat was moving.” So begins a recent paper by Zamir, Rongish, and Little, published in PLoS Biology (Zamir et al., 2008). Recent time-lapse microscopy studies have described the cellular movements that result in chicken primitive streak (PS) formation (the PS is the organizing center for gastrulation) (Chuai et al., 2006; Voiculescu et al., 2007). However, the movements of cells and their ECM had not been simultaneously visualized during PS formation. Zamir and colleagues analyzed the relative movements of epiblast cells compared to the meshwork of Fn fibrils they termed SE ECM (the mesh of Fn fibrils that lie subjacent to the epiblastic epithelium). Their findings suggest that the movement of epiblast cells and the SE ECM is highly correlated. This methodology allowed them to also analyze cell autonomous movements (movements of cells relative to the SE ECM). Interestingly, although there was a small degree of cell autonomous movements, they resembled more of a “random walk” than a coordinated morphogenetic event. This exciting and highly informative study should change the way that developmental biologists investigate cell migration in different contexts. In particular, as noted by the authors, the concept of extracellular chemotactic gradients becomes exponentially more complex when it is considered that the ECM itself may move along with the cells.

SUMMARY

The studies described herein, along with many other fantastic experiments that were not discussed due to space constraints, clearly show that dynamic modulation of both the ECM and cell adhesion to the ECM are critical for development and physiology. These studies also highlight the complexity of cell–ECM adhesion complexes. CMACs contain hundreds of proteins, most of which are regulated by post-translational modification. At least two major technical issues currently hinder progress within the field. One is the paucity of technology for high resolution in vivo imaging of CMAC components. Some laboratories have developed the technology for in vivo imaging of Fn, as described above. This is a critical step, but more remains to be done. Multiple CMACs and their post-translational modifications need to be imaged and manipulated in vivo. A recent, exciting paper describes a method for visualizing glycans in vivo during zebrafish development (Laughlin et al., 2008). One can envision refinement of this tool for visualization of proteoglycans in synthesis and assembly into three-dimensional scaffolds in vivo. This is an area of rapidly developing technology and also rapid incorporation of excellent tools developed in vitro for in vivo use. The other major hindrance in understanding of the dynamic modulation of CMAC function in vivo is a lack of quantitative readouts of cell–ECM signaling. Until quantitative methods are available to assess the type and amount of cell–ECM signaling, it will be quite difficult to synthesize an integrative view of CMAC function in development and physiology. Finally, in this era of deep sequencing, proteomics, and chemical genetic screens, it is important to remember that understanding the dynamics and mechanics of cell adhesion to the matrix in vivo is fundamentally required for true progress towards amelioration of most diseases. In this sense, cell–ECM adhesion can be thought of as fractal: detail is found at all scales analyzed.

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