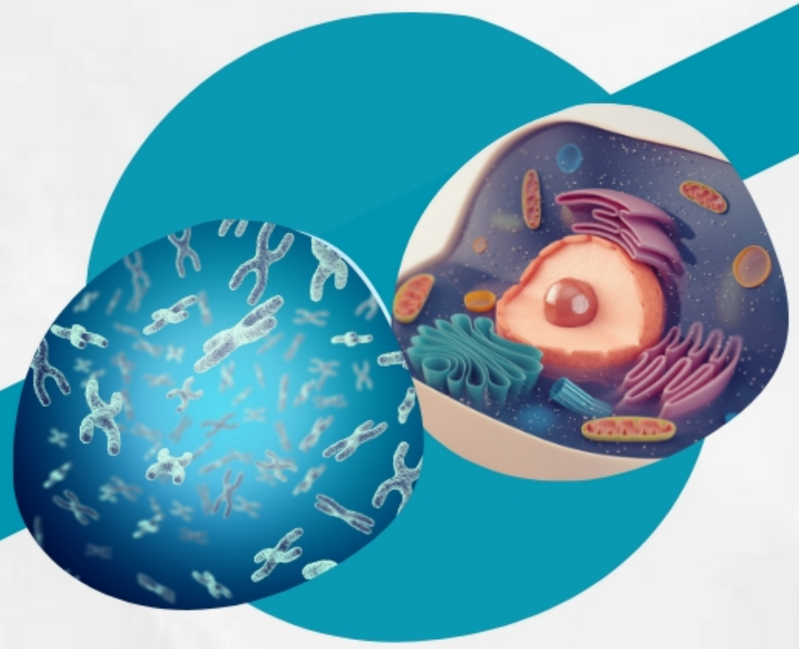
Sheets

Cell biology

7.2-7.3



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REMEMBER: Stem cells are unique, unspecialized cells in multicellular organisms that can divide indefinitely (self-renew) and differentiate into specialized cell types.

7.2 Engineering Linkage: Organoids

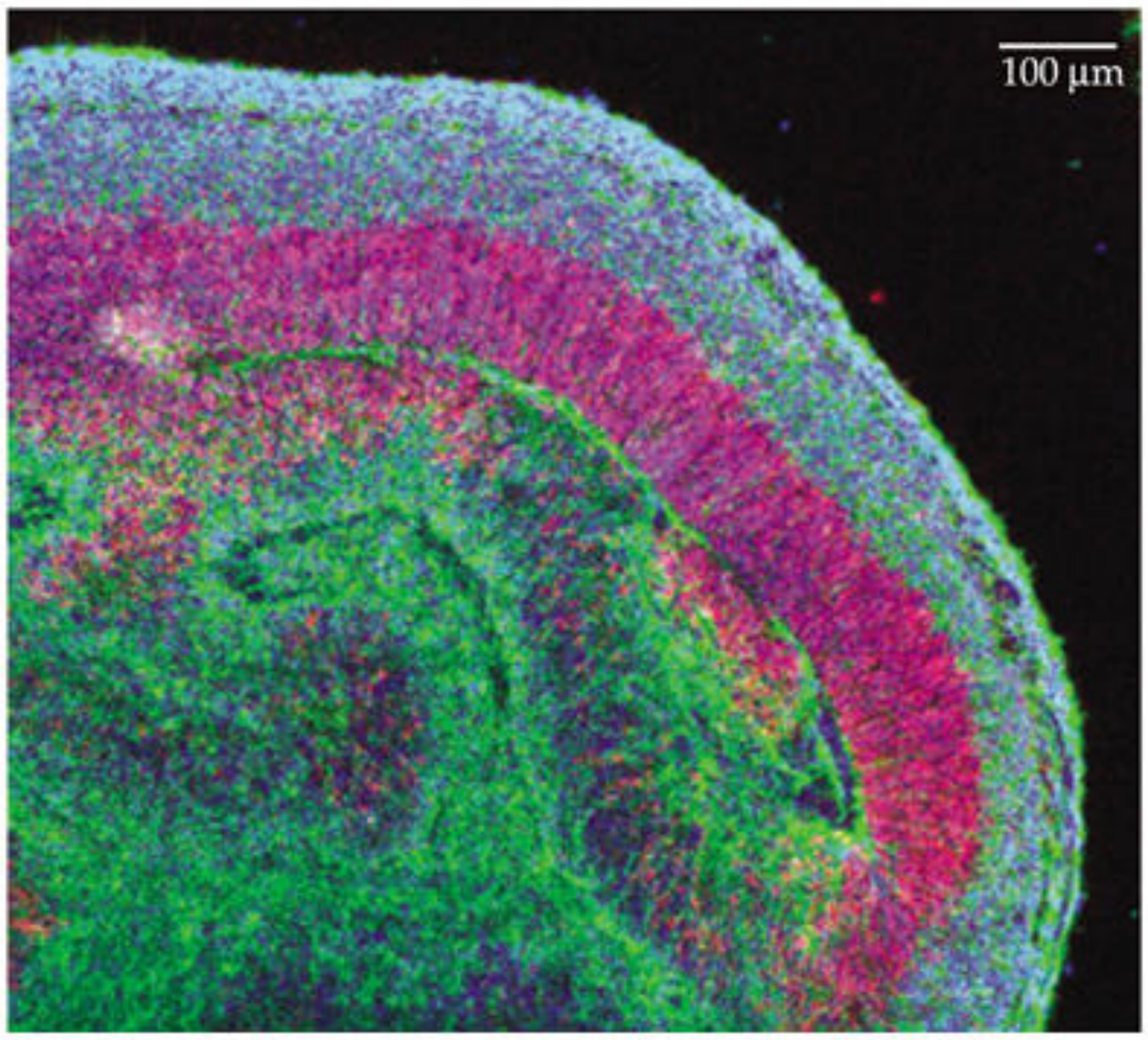
The idea of growing human organs in a laboratory might sound like the stuff of science fiction. In fact, the engineering of organoids, three-dimensional (3D) cellular structures that resemble organs in both development and organization, has made significant advances in recent years and has numerous promising applications in the lab as well as the clinic.

Several different methods support the growth of 3D cell cultures. Cells are typically derived from stem cells, which can be tissue-specific stem cells from a mature organism (such as stem cells from adult intestinal crypts), embryonic stem cells, or induced pluripotent stem cells. These cells are then grown in a thick, gel-like medium containing a complex mixture of extracellular matrix proteins and other components that encourage cellular growth (the most well known is sold as Matrigel™). Depending on the organ that is being recapitulated, different cocktails of growth factors and additional extracellular matrix components must be added during organoid development. The end result is a small clump of tissue that can often approximate a full-sized organ in different ways.

Group

In recent years, researchers have made progress in developing organoids from a variety of different tissues, including the intestine, stomach, pancreas, optic cup, and brain. How closely these organoids match with the parental tissue varies, but typically organoids can recapitulate many aspects of 3D architecture, tissue development, and cellular differentiation. In the case of intestinal organoids, for example, villus-like epithelia with

crypt-like domains and a central lumen are apparent. Brain organoids develop layers of neuronal cells that mimic the organization of the cortex (see **Figure 7.13**).



Source: From Figure 2, Akkerman and Defize. Dawn of the organoid era. *Bio-Essays*, 2017. Licensed under CC BY 4.0, https://onlinelibrary.wiley.com/doi/pdf/10.1002/bies.201600244.

FIGURE 7.13 Fluorescence microscopy image of a brain organoid. Neurons, labeled green, are enriched in the outermost layer of the cortex, while neural stem cells are labeled in pink.

Having "organs in a dish" provides a multitude of opportunities for drug developers, clinicians, and scientists. Organoids have been used to[1] study diseases and test new drug therapies. For example, brain organoids were used to study microcephaly caused by the Zika virus, and intestinal organoids created from patients have been used to model and treat cystic fibrosis. Since organoids can be derived from individual adults,[2] it also holds promise for the development of personalized medicine. In the near future, clinicians may be able to grow a series of organoid cultures as a "body on a chip" that can be used for individualized and high-throughput testing of potential therapeutics.

Review

1. Describe how an organoid culture is grown in the lab.
2. How might organoid cultures be used for personalized medicine?

7.3 Interactions of Cells with Extracellular Materials

As you learned in Section 7.1, components of the ECM, such as fibronectin, laminin,

proteoglycans, and collagen, are capable of binding to receptors situated on the cell surface (as in Figure 7.5). The most important family of receptors that attach cells to their extracellular microenvironment is the integrins.

Integrins

Integrins, a family of membrane proteins found only in animals, play a key role in integrating the extracellular and intracellular environments. On the outer side of the plasma membrane, integrins bind to a remarkably diverse array of molecules (ligands) that are present in the extracellular environment. On the intracellular side of the membrane, integrins interact either directly or indirectly with dozens of different proteins to influence the course of events within the cell. Integrins are composed of two membrane-spanning polypeptide chains, an α chain and a β chain, that are noncovalently linked. Eighteen different α subunits and eight different β subunits have been identified. Although theoretically more than a hundred possible pairings of α and β subunits could occur, only about two dozen different integrins have been identified on the surfaces of cells, each with a specific distribution within the body. Whereas many subunits occur in only a single integrin heterodimer, the β_1 subunit is found in 12 different integrins and the α_V subunit in 5 of them (see **Table 7.1**). Most cells possess a variety of different integrins; conversely, most integrins are present on a variety of different cell types.

Two dozen is equal to 24.

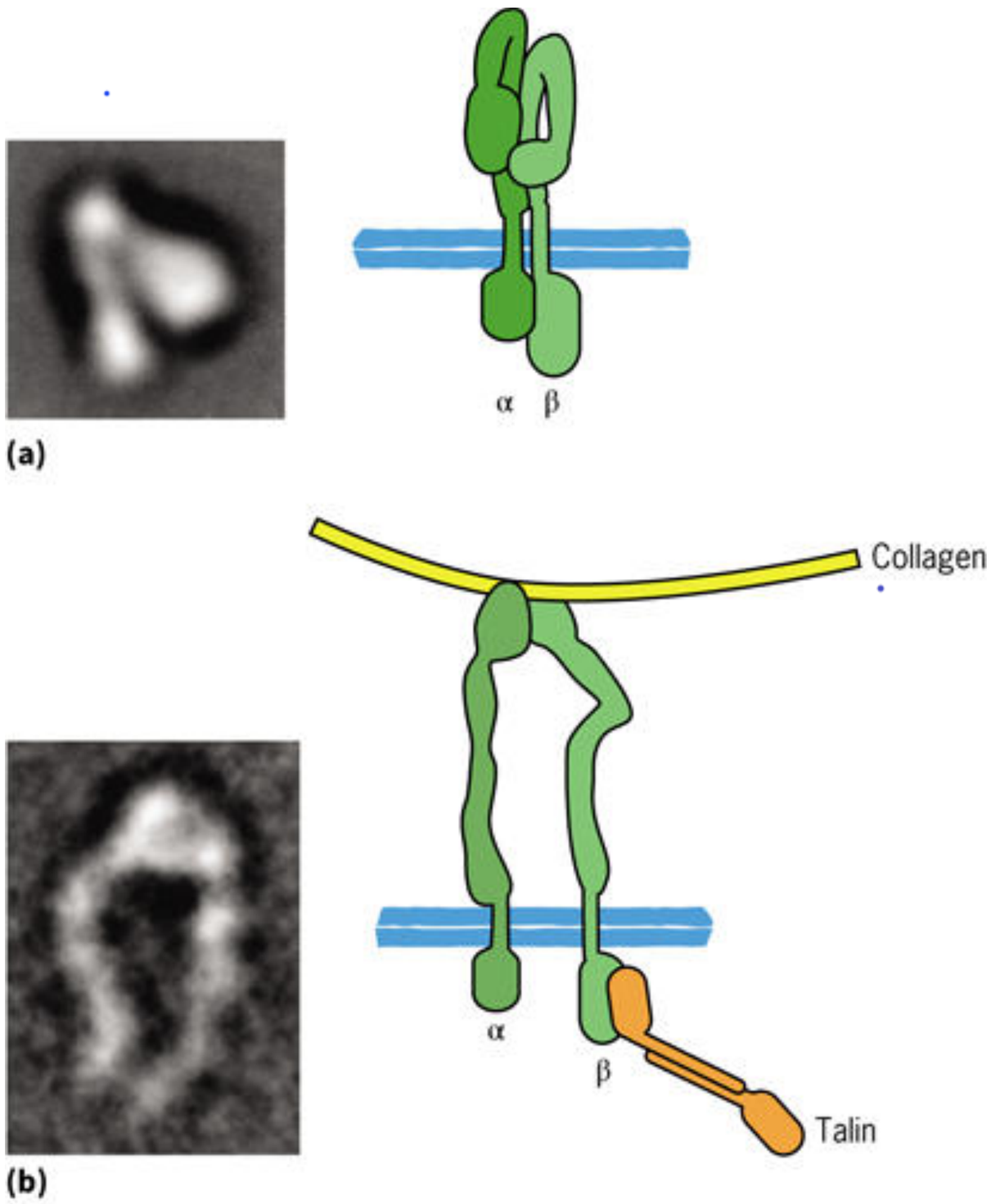
TABLE 7.1

Classification of Integrin Receptors Based on Recognition of RGD Sequences

Source: From S. E. D'Souza, M. H. Ginsberg, E. F. Plow, *Trends Biochemical Sciences* 16:249, 1991. ©1991. Reprinted with permission from Elsevier.

RGD Recognition		Non-RGD Recognition	
Integrin receptor	**Key ligands**	**Integrin receptor**	**Key ligands**
$\alpha_3\beta_1$	Fibronectin	$\alpha_1\beta_1$	Collagen
$\alpha_5\beta_1$	Fibronectin	$\alpha_2\beta_1$	Collagen
$\alpha_v\beta_1$	Fibronectin		Laminin
		$\alpha_3\beta_1$	Collagen
$\alpha_{IIb}\beta_3$	Fibronectin		Laminin
	von Willebrand factor	$\alpha_4\beta_1$	Fibronectin
	Vitronectin		VCAM
	Fibrinogen	$\alpha_6\beta_1$	Laminin
$\alpha_v\beta_3$	Fibronectin	$\alpha_L\beta_2$	ICAM-1
	von Willebrand factor		ICAM-2
	Vitronectin	$\alpha_M\beta_2$	Fibrinogen
$\alpha_v\beta_5$	Vitronectin		ICAM-1
$\alpha_v\beta_6$	Fibronectin		

Electron microscopic observations of integrin molecules beginning in the late 1980s suggested that the two subunits are oriented to form a globular extracellular head connected to the membrane by a pair of elongated "legs" (as depicted in Figure 7.5). The legs of each subunit extend through the lipid bilayer as a single transmembrane helix and end in a small cytoplasmic domain of about 20 to 70 amino acids.[1] The first X-ray crystallographic structure of the extracellular portion of an integrin was published in 2001 and displayed a highly unexpected feature. Rather than "standing upright" as predicted, the integrin $\alpha_v\beta_3$ was bent dramatically at the "knees" so that the head faces the outer surface of the plasma membrane (**Figure 7.14**). To understand the significance of this bent structure, we need to explore the properties of integrins.



Source: Electron micrographs from Junichi Takagi et al., *Cell* 110:601, 2002; with permission from Elsevier

FIGURE 7.14 Integrin conformations. (a) Schematic drawing of a complete integrin in the bent, inactive conformation, and a corresponding electron micrograph of the extracellular portion of a similar molecule. (b) Schematic drawing of an integrin in an active conformation and bound to a ligand, in this case talin. Binding of talin to the small cytoplasmic domain of the β subunit induces a separation of the two subunits and conversion to the active conformation. Activated integrins typically become clustered as the result of interactions of their cytoplasmic domains with the underlying cytoskeleton as indicated in Figure 7.17c. The extracellular ligand, in this case a collagen fiber, binds to both of the subunits in the head region of the activated integrin dimer. Note that conformations intermediate between those shown here may also exist and exhibit reduced ligand-binding. in this case the subunit are close to each other.

A body of evidence strongly suggests that the bent conformation of an integrin shown in Figure 7.14*a* corresponds to the inactive state of the protein, which is incapable of binding a ligand. In fact, when an $\alpha_V\beta_3$ integrin containing a bound ligand is analyzed, the integrin no longer exhibits the bent structure but is present instead in an upright conformation as illustrated in Figure 7.14*b*. The ligand is bound to the head of the integrin in a cleft where the α and β subunits come together If the bent and upright structures represent the inactive and active states of an integrin, respectively, then it is important to consider what type of stimulus

is capable of triggering such a remarkable transformation in protein conformation.

In the bent, inactive conformation, the transmembrane domains of the two subunits are in close proximity, apparently held together by noncovalent interactions between residues of the two helices. The cytoplasmic domains of integrins bind a wide array of proteins; one of these proteins, called talin, causes separation of the α and β subunits, as shown in the structure of the activated integrin in Figure 7.14*b* Mutations in talin that block its interaction with β-integrin subunits also prevent activation of integrins and adhesion to the ECM.

When talin (a protein inside the cell) binds an integrin, it separates the tails and transmembrane domains, activating the integrin to bind ligands. Clustering of activated integrins further strengthens cell-ligand interactions. This process is known as inside-out signaling.

Integrins have been implicated in two major types of activities: adhesion of cells to their substratum (or to other cells) and transmission of signals between the external environment and the cell interior. We have already discussed the transmission of signals in an inside-out direction, which primarily affects the binding properties of the integrins. Signaling can also occur in the opposite direction, a phenomenon known as “outside-in” signaling. The binding of the extracellular domain of an integrin to a ligand, such as fibronectin or collagen, can induce a conformational change at the opposite, cytoplasmic end of the integrin, especially its β subunit. Changes at the cytoplasmic end can, in turn, alter the way the integrin interacts with a host of different cytoplasmic proteins, modifying their activity. For example, outside-in signals can induce a conformational change in talin on the inside of the membrane, initiating a cascade of events leading to the polymerization of actin filaments of the cytoskeleton. Binding of integrins to an extracellular ligand can also trigger the activation of cytoplasmic protein kinases, such as FAK and Src (see Figure 7.17*c*). These kinases can then phosphorylate other proteins, initiating a chain reaction. In some cases, the chain reaction leads all the way to the nucleus, where a specific group of genes may be activated.

Outside-in signals transmitted by integrins (and other cell surface molecules) can influence many aspects of cell behavior, including differentiation, motility, growth, and even the survival of the cell. The influence of integrins on cell survival is best illustrated by comparing normal and malignant cells. Most malignant cells are capable of growing while suspended in a liquid culture medium. Normal cells, in contrast, can only grow and divide if they are cultured on a solid substratum; if they are placed in suspension cultures, they die. Normal cells are thought to die in suspension culture because their integrins are not able to interact with extracellular substrates and, as a result, are not able to transmit life-saving signals to the interior of the cell. When cells become malignant, their survival no longer depends on integrin binding.

Table 7.1 lists a number of known integrins and the key extracellular ligands to which they bind. Because individual cells may express a variety of different integrins on their cell surface, such cells are capable of binding to a variety of different extracellular components (Figure 7.5). Despite the apparent overlap seen in Table 7.1, most integrins do appear to have unique functions, as knockout mice (Section 18.16) that lack different integrin subunits exhibit distinct phenotypes. For example, α_8 knockouts show kidney defects, α_4 knockouts exhibit

heart defects, and α_5 knockouts display vascular defects.

Many of the extracellular proteins that bind to integrins do so because they contain the amino acid sequence arginine-glycine-aspartic acid (or, in abbreviated amino acid nomenclature, RGD). This tripeptide is present in the cell-binding sites of proteoglycans, fibronectin, laminin, and various other extracellular proteins. The cell-binding domain of fibronectin, with its extended RGD-containing loop, is shown in Figure 7.10*a*.

The discovery of the importance of the RGD sequence has opened the door to new avenues for treatment of medical conditions that involve receptor-ligand interactions. When the wall of a blood vessel is injured, the damaged region is sealed by the controlled aggregation of blood platelets, which are nonnucleated cell fragments that circulate in the blood. When it occurs at an inappropriate time or place, the aggregation of platelets can form a potentially dangerous blood clot (*thrombus*) that can block the flow of blood to major organs, one of the leading causes of heart attack and stroke. The aggregation of platelets requires the interaction of a platelet-specific integrin ($\alpha_{IIb}\beta_3$) with soluble RGD-containing blood proteins, such as fibrinogen and von Willebrand factor, which act as linkers that hold the platelets together (**Figure 7.15a**). Experiments with animals had indicated that RGD-containing peptides can inhibit blood clot formation by preventing the platelet integrin from binding to blood proteins (Figure 7.15*b*). These findings led to the design of a new class of antithrombotic agents (Aggrastat and Integrelin) that resemble the RGD structure but bind selectively to the platelet integrin. Both of these drugs are based on the structures of compounds present in snake venom. A specific antibody (ReoPro) directed against the RGD binding site of $\alpha_{IIb}\beta_3$ integrins can also prevent blood clots in certain patients undergoing high-risk vascular surgeries. A different integrin-targeting antibody (Tysabri) is prescribed for treatment of multiple sclerosis (see The Human Perspective, Chapter 17).

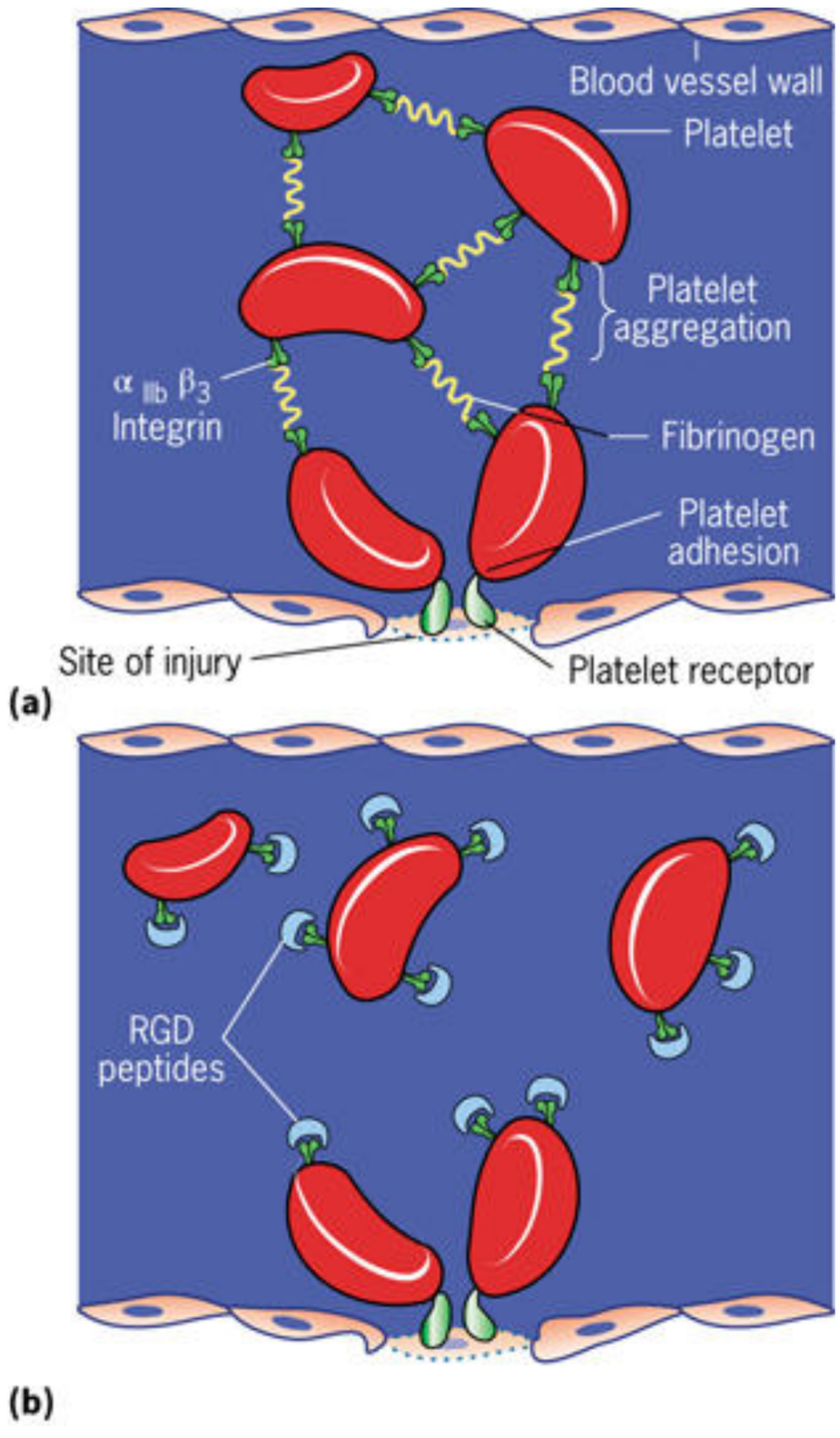


FIGURE 7.15 The role of integrins in platelet aggregation. (a) Blood clots form when platelets adhere to one another through fibrinogen bridges that bind to the platelet integrins. (b) The presence of synthetic RGD peptides can inhibit blood clot formation by competing with fibrinogen molecules for the RGD-binding sites on platelet $\alpha_{IIb}\beta_3$ integrins. Nonpeptide RGD analogues and anti-integrin antibodies can act in a similar way to prevent clot formation in high-risk patients.

The cytoplasmic domains of integrins contain binding sites for a variety of cytoplasmic proteins, including several that act as adaptors that link the integrin to actin filaments of the cytoskeleton (see Figure 7.17). The role of integrins in making the connection between the ECM and the cytoskeleton is best seen in two specialized structures: focal adhesions and hemidesmosomes.

Focal Adhesions

It is much easier to study the interaction of cells with the bottom of a culture dish than with an extracellular matrix inside an animal. Consequently, much of our knowledge of cell–matrix interactions has been obtained from the study of cells adhering to various substrates in vitro. The stages in the attachment of a cultured cell to its substratum are shown in **Figure 7.16**. At first, the cell has a rounded morphology, as is generally true of animal cells

suspended in aqueous medium. Once the cell makes contact with the substratum, it sends out projections that form increasingly stable attachments. Over time, the cell flattens and spreads itself out on the substratum.

The surface where cells will be cultured is coated with ECM materials, enabling cell attachment. Cells also construct and secrete ECM externally. This is essential for their survival.

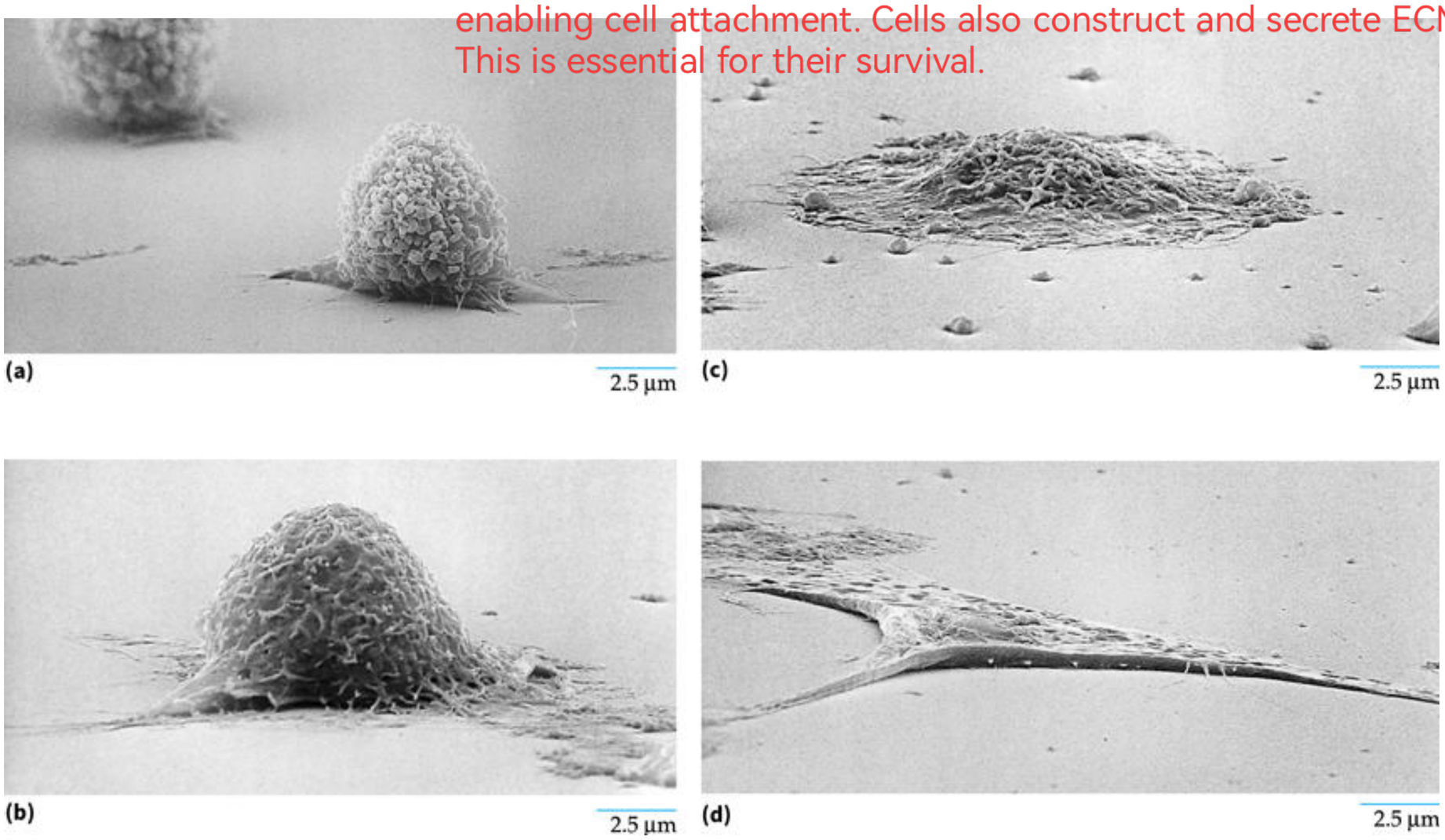
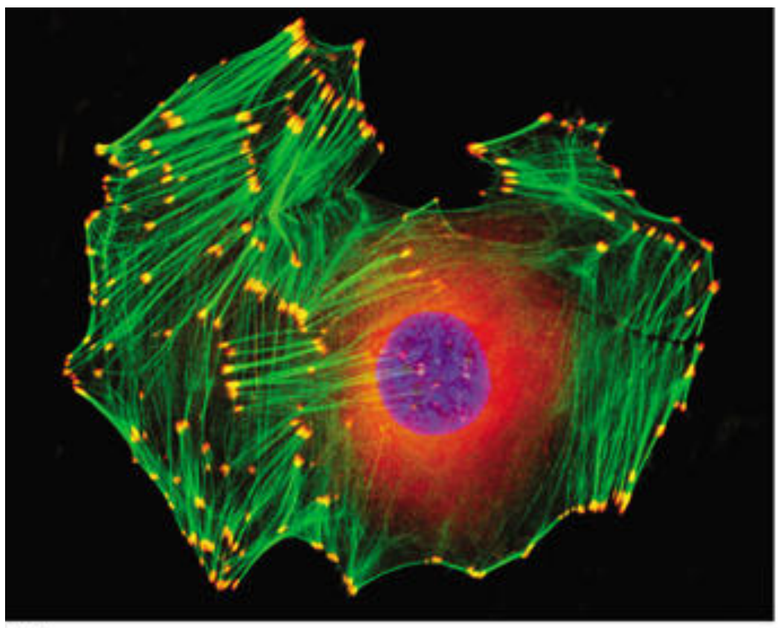
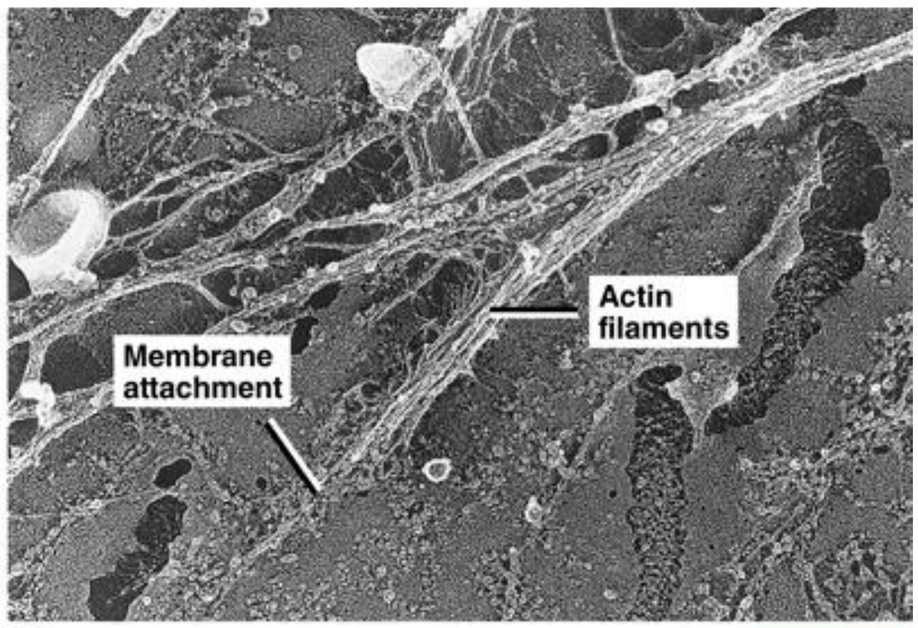


FIGURE 7.16 Steps in the process of cell spreading. Scanning electron micrographs showing the morphology of mouse fibroblasts at successive times during attachment and spreading on glass coverslips. Cells were fixed after (a) 30 minutes, (b) 60 minutes, (c) 2 hours, and (d) 24 hours of attachment.

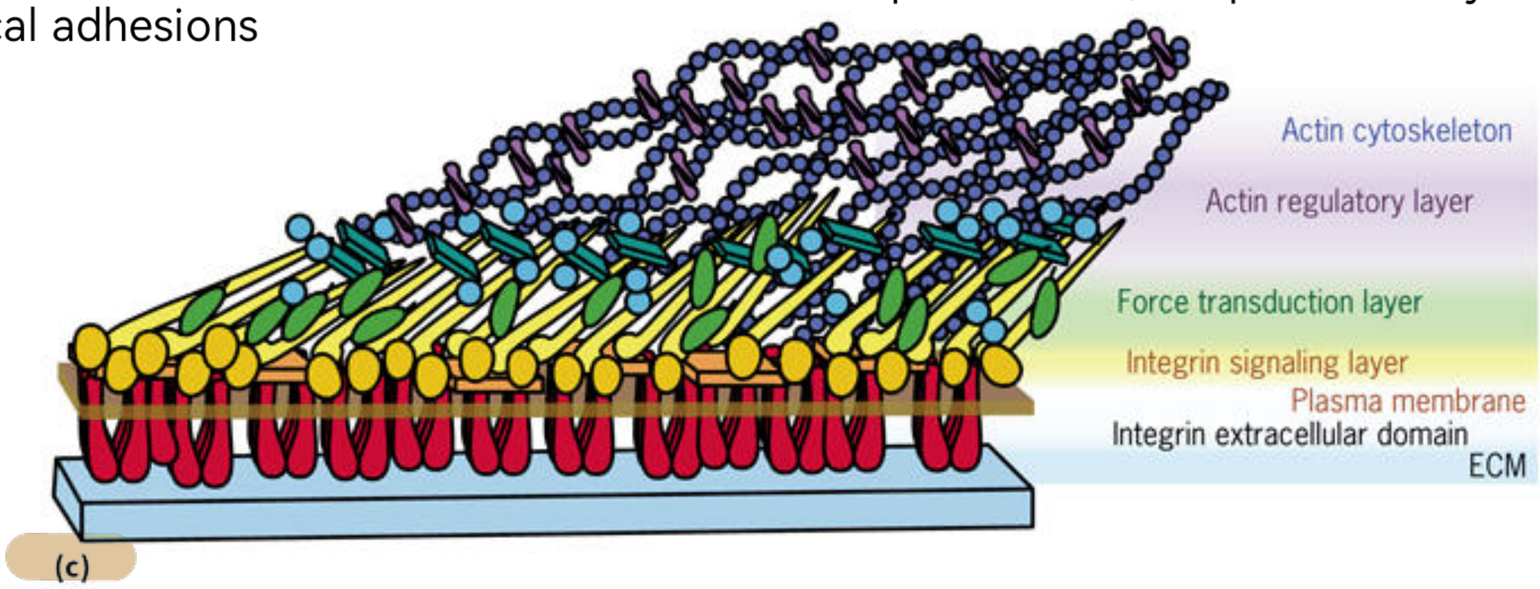
When fibroblasts or epithelial cells spread onto the bottom of a culture dish, the lower surface of the cell is not pressed uniformly against the substratum. Instead, the cell is anchored to the surface of the dish only at scattered, discrete sites, called **focal adhesions** (**Figure 7.17**). Focal adhesions are dynamic structures that can be rapidly disassembled if the adherent cell is stimulated to move or enter mitosis. The plasma membrane of a focal adhesion contains large clusters of integrins. The cytoplasmic domains of the integrins are connected to actin filaments of the cytoskeleton through stratified layers of adaptor proteins (e.g., talin, α-actinin and vinculin) (Figure 7.17c). Focal adhesions play a key role in cell locomotion, during which the integrins develop transient interactions with extracellular materials (see Figure 9.67).



(a) Cultured cell: actin filaments (gray-green), integrins (red); sites of focal adhesions



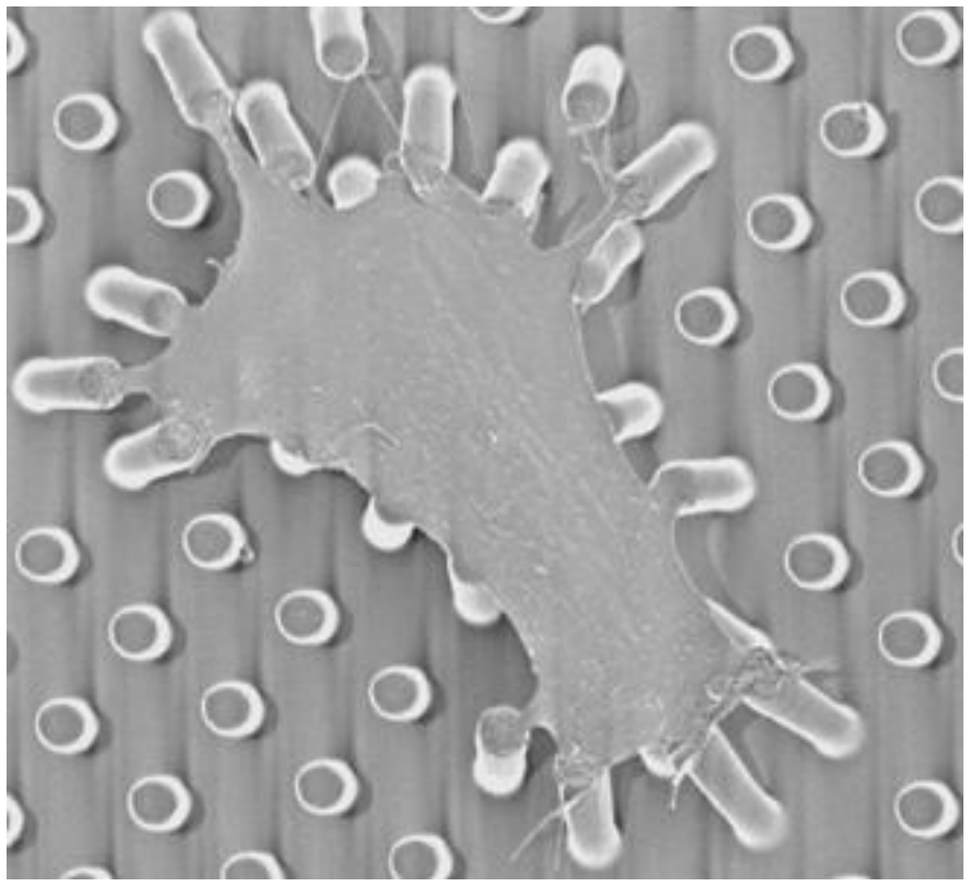
(b) Amphibian cell processed for quick-freeze, deep-etch analysis



Source: (*a*) From Molecular expressions.com at Florida State University; (*b*) ©1993 Steven J. Samuelssonet al. Originally published in *The Journal of Cell Biology* http://doi.org/10.1083/jcb.122.2.485. (*c*) From P. Kanchanawong et al., Reprinted by permission from Macmillan Publishers Ltd: *Nature* 468:580–584, 2010.

FIGURE 7.17 Focal adhesions are sites where cells adhere to their substratum and transmit signals in both directions across the plasma membrane. (a) This cultured cell has been stained with fluorescent antibodies to reveal the locations of actin filaments (green) and integrins (red). The integrins are localized in small patches that correspond to the sites of focal adhesions. (b) The cytoplasmic surface of a focal adhesion of a cultured amphibian cell is shown here after the inner surface of the membrane was processed for quick-freeze, deep-etch analysis. Bundles of actin filaments are seen to associate with the inner surface of the membrane in the region of a focal adhesion. (c) Schematic drawing of a focal adhesion showing the interactions of integrin molecules with other proteins on both sides of the lipid bilayer. The binding of extracellular ligands, such as collagen and fibronectin, is thought to induce conformational changes in the cytoplasmic domains of the integrins that cause the integrins (red) to become linked to actin filaments of the cytoskeleton. Linkages with the cytoskeleton lead, in turn, to the clustering of integrins at the cell surface. Linkages with the cytoskeleton are mediated by various actin-binding proteins, such as talin (shown in yellow) and α-actinin (purple), that bind to the β subunit of the integrin. During the process of focal adhesion formation, talin undergoes a conformational change that exposes binding sites on talin's rod domain. The binding of vinculin (light green) to these exposed sites promotes the assembly of additional actin filaments. The cytoplasmic domains of integrins are also associated with protein kinases, such as FAK (focal adhesion kinase, shown in orange) and Src. The attachment of the integrin to an extracellular ligand can activate these protein kinases and start a chain reaction that transmits signals throughout the cell. The association of myosin molecules with the actin filaments can generate traction forces that are transmitted to sites of cell–substrate attachment.

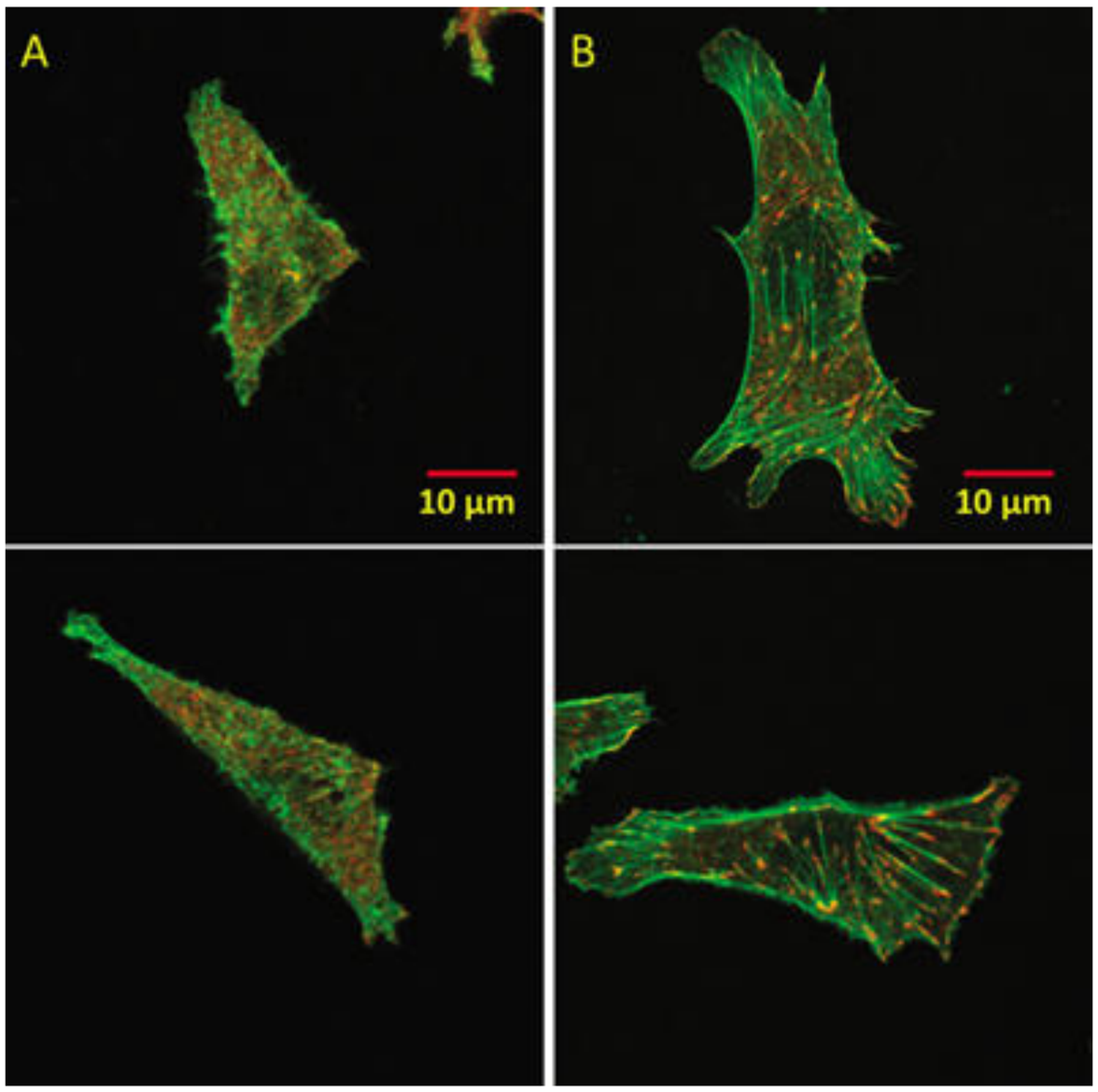
Focal adhesions are capable of creating mechanical forces or responding to such forces. These properties might be expected from a structure that contains actin and myosin, two of the cell's major contractile proteins. **Figure 7.18** shows a fibroblast lying on a bed of fibronectin-coated posts. The posts are flexible and capable of being moved independently of one another. It can be seen in this micrograph that the tips of the posts are being deflected as a result of pulling forces exerted by the cell's focal adhesions.



Source: From John L. Tan, et al., *PNAS* 100:1484, 2003, fig 2d, courtesy of Christopher S. Chen/PNAS vol. 105 no. 49/Copyright (2003) National Academy of Sciences, U.S.A.

FIGURE 7.18 Experimental demonstration of forces exerted by focal adhesions. Scanning-electron micrograph of a smooth muscle cell sitting atop a bed of flexible (elastomeric) micro-posts with tips that had been coated with fibronectin. The attached cell is seen deflecting the position of multiple posts. The degree of movement of a particular post reflects the magnitude of the traction forces exerted by the cell on that post.

Acting in the opposite direction, mechanical forces applied to the surfaces of cells can be converted by focal adhesions into biochemical signals in the cytoplasm. This process of signal conversion is described as *mechanotransduction*, and the focal adhesion is acting as a *mechanosensor* by recognizing the physical properties of the environment. The ability of cells to respond to physical forces is important in many cellular behaviors and can be illustrated by a study in which cells were allowed to bind to beads that had been covered with a coating of fibronectin. When the membrane-bound beads were pulled by an optical tweezer, the mechanical stimulus was transmitted into the cell interior where it generated a wave of Src kinase activation. The strength of an integrin–ligand bond was measured to be on the order of 40 piconewtons (pN), based on an experiment in which an integrin ligand was immobilized on a surface using different molecular tethers that ruptured at different forces. As shown in **Figure 7.19**, a tether of 56 pN allowed the cell to form strong focal adhesions and actin stress fibers, while a tether of 43 pN did not. Mechanotransduction is thought to be mediated by conformational changes in some of the adaptor proteins, such as talin, which are induced by stretching. Such conformational changes can expose important, previously hidden binding sites in these proteins, allowing additional protein molecules to be recruited to the adhesion complex.



Source: From Wang et al., Science 340: 6135:991–4, 2013. Reprinted with permission from AAAS.

FIGURE 7.19 Measuring the force necessary to activate integrins. In this experiment, an integrin ligand was attached to a glass substrate using a tether that would rupture once a specific force is applied. Mammalian cells were allowed to spread on the glass, and the cells were stained for actin (green) and a focal adhesion marker (vinculin, red). When tethers of a rupture strength of 43 piconewtons were used, integrins were not activated, and focal adhesions did not form. However, tethers of a rupture strength of 56 piconewtons resulted in integrin activation, as seen through the formation of focal adhesions (right).

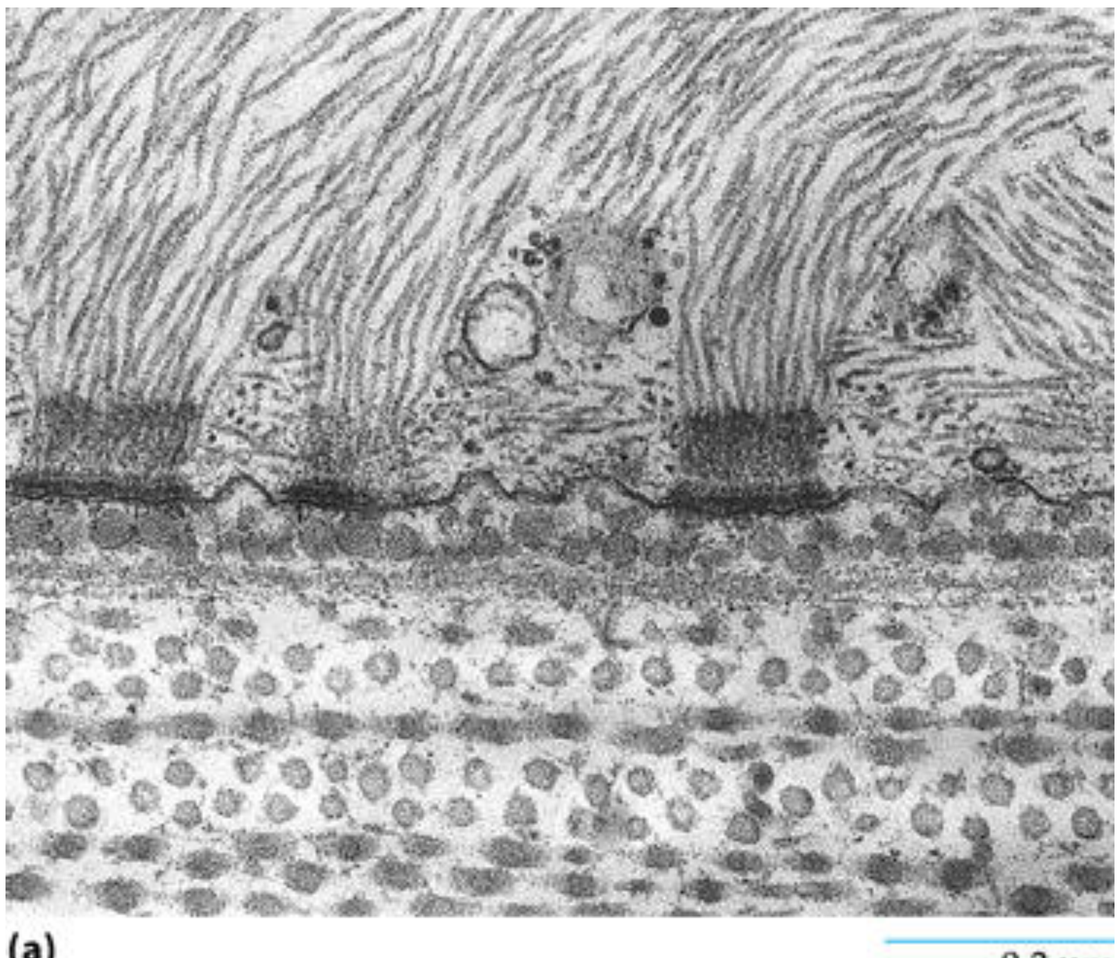
Figure 7.17c illustrates how the pull of a fibronectin or collagen molecule is able to activate protein kinases, such as FAK or Src, which would then phosphorylate various substrates. Activation of these protein kinases can transmit signals throughout the cell, including the cell nucleus, where they can promote changes in gene expression. Activation of either FAK or Src can dramatically alter cell behavior. The importance of the physical properties of a cell's environment in influencing cellular behavior is illustrated by a study in which mesenchymal stem cells (MSCs) derived from adult bone marrow were grown on substrates of varying elasticity (or stiffness). When these MSCs were grown on a soft, pliable substratum, such as might be encountered by a cell within the developing brain, the MSCs differentiated into nerve cells. When grown on a substratum of greater stiffness, these same cells differentiated into muscle cells. Finally, when grown on even stiffer substrates, such as those that might be home to cells growing in a skeletal tissue such as cartilage or bone, the MSCs differentiated into osteoblasts, which give rise to bone cells.

Any pull or push on the ECM activates the kinase "mechanical receptor."

Hemidesmosomes

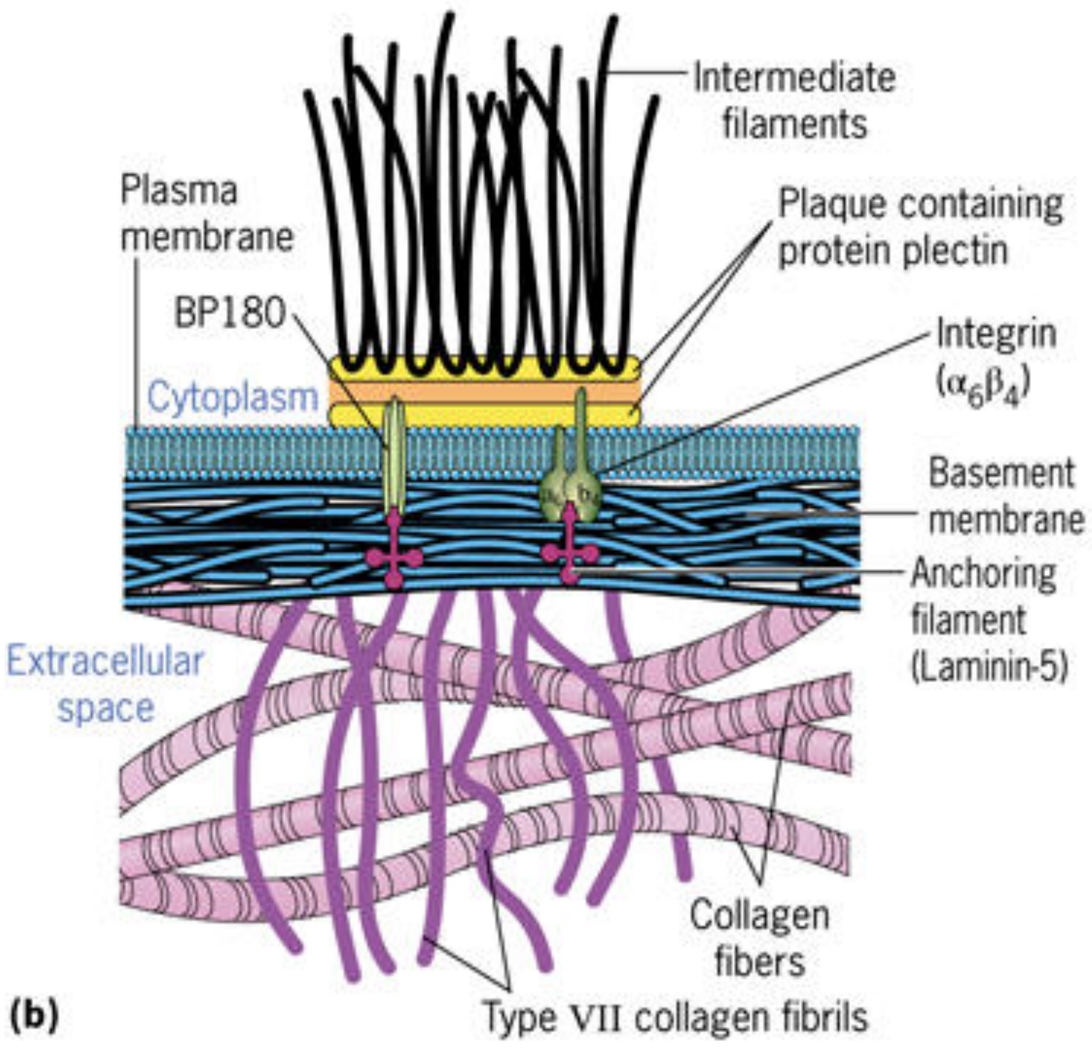
Focal adhesions are most commonly seen in cells grown in vitro, although similar types of

adhesive contacts are found in certain tissues, such as muscle and tendon. Within the body, the tightest attachment between a cell and its extracellular matrix is seen at the basal surface of epithelial cells, where the cells are anchored to the underlying basement membrane by a specialized adhesive structure called a **hemidesmosome** (Figures 7.1 and 7.20). Hemidesmosomes contain a dense plaque on the inner surface of the plasma membrane with filaments coursing outward into the cytoplasm (**Figure 7.20a**). Unlike the filaments of focal adhesions, which consist of actin, the filaments of the hemidesmosome are thicker and consist of the protein keratin. Keratin-containing filaments are classified as intermediate filaments, which serve primarily in a supportive function (as discussed in detail in Section 9.7). The keratin filaments of the hemidesmosome are linked to the extracellular matrix by membrane-spanning integrins, including $\alpha_6\beta_4$ (Figure 7.20*b*). Like their counterparts in focal adhesions, these integrins also transmit signals from the ECM that affect the shape and activities of the attached epithelial cells.



(a)

0.3 μm



(b) Type VII collagen fibrils

Source: (*a*) ©1966 Douglas E. Kelly Originally published in *The Journal of Cell Biology* http://doi.org/10.1083/jcb.28.1.51

FIGURE 7.20 Hemidesmosomes. Hemidesmosomes are differentiated sites at the basal surfaces of epithelial cells where the cells are attached to the underlying basement membrane. (a) Electron micrograph of several hemidesmosomes showing the dense plaque on the inner surface of the plasma membrane and the intermediate filaments projecting into the cytoplasm. (b) Schematic diagram showing the major components of a hemidesmosome connecting the epidermis to the underlying dermis. The $\alpha_6\beta_4$ integrin molecules of the epidermal cells are linked to cytoplasmic intermediate filaments by a protein called plectin that is present in the dark-staining plaque and to the basement membrane by anchoring filaments of a particular type of laminin. A second transmembrane protein (BP180) is also present in hemidesmosomes. The collagen fibers are part of the underlying dermis.

The importance of hemidesmosomes is revealed by a rare disease, *bullous pemphigoid*, in which individuals produce antibodies that recognize proteins present in these adhesive structures. Autoimmune disorders, diseases caused by production of antibodies directed against one's own tissues (i.e., autoantibodies), are responsible for a wide variety of conditions. In this case, the presence of autoantibodies causes the lower layer of the epidermis to lose attachment to the underlying basement membrane (and thus to the underlying connective tissue layer of the dermis). The leakage of fluid into the space beneath the epidermis results in severe blistering of the skin. A similar inherited blistering disease, *epidermolysis bullosa*, can occur in patients with genetic alterations in any one of a number of hemidesmosomal proteins, including the α_6 or β_4 integrin subunit, collagen VII, or laminin-5.

Review

1. How are integrins able to link the cell surface with materials that make up the ECM? How do the inactive and active structures of integrins differ from one another structurally and functionally? What is the significance of the presence of an RGD motif in an integrin ligand?
2. How can a cell-surface protein be involved in both cell adhesion and transmembrane signal transduction?
3. What is the difference between inside-out and outside-in signaling? What is the importance of each to cell activities?
4. List two characteristics that distinguish hemidesmosomes from focal adhesions.