Review

Integrins Control Vesicular Trafficking; New Tricks for Old Dogs

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Integrins are transmembrane receptors that transduce biochemical and mechanical signals across the plasma membrane and promote cell adhesion and migration. In addition, integrin adhesion complexes are functionally and structurally linked to components of the intracellular trafficking machinery and accumulating data now reveal that they are key regulators of endocytosis and exocytosis in a variety of cell types. Here, we highlight recent insights into integrin control of intracellular trafficking in processes such as degranulation, mechanotransduction, cell–cell communication, antibody production, virus entry, Toll-like receptor signaling, autophagy, and phagocytosis, as well as the release and uptake of extracellular vesicles. We discuss the underlying molecular mechanisms and the implications for a range of pathophysiological contexts, including hemostasis, immunity, tissue repair, cancer, and viral infection.

Highlights

Integrin adhesion complexes control polarized targeting of the intracellular trafficking machinery via microtubules.

Integrin adhesions are exocytic hubs for a variety of vesicles, including lytic and dense granules, lysosome-related organelles, and biosynthetic vesicles.

Integrin-dependent adhesion and signaling is required for degranulation of platelets and leukocytes and controls hemostasis and immunity.

Specialized adhesion complexes containing integrin αvβ5 and clathrin are sites of frustrated endocytosis and hubs for mechanotransduction.

Integrin control of endocytosis regulates Toll-like receptor signaling and autophagy in immune cells.

Integrins control intercellular communication and viral transfer through extracellular vesicles.

Integrins and Functions of Adhesion Complexes

The integrin family consists of 24 heterodimeric transmembrane receptors assembled from 18 α-subunits and eight β-subunits. Integrins recognize a plethora of proteins either on the surface of other cells or in the **extracellular matrix (ECM)** (see Glossary) and are essential for cell adhesion and spreading, migration, and ECM organization [1]. Furthermore, integrins transduce mechanical and biochemical signals across the plasma membrane and promote cell proliferation and survival [1]. The cytoplasmic tails of integrins regulate their affinity for ligands, association with the cytoskeleton, and the assembly of adhesion complexes, which contain a variety of structural and adapter proteins and in addition serve as 'hubs' for signaling pathways [2–4]. Most integrin adhesion complexes, including **focal adhesions (FAs)**, fibrillar adhesions, **immunological synapses**, and podosomes, are linked to the actin cytoskeleton via a number of proteins such as talin, which bridges integrin β-tails with actin filaments (Figure 1, Key Figure) [1–4]. Accumulating evidence indicates that integrin adhesion complexes also interact with **microtubules**, thus linking them to the intracellular trafficking machinery regulating **exocytosis** [5,6]. Furthermore, a new type of adhesion complex called **flat clathrin lattice (FCL)** has recently emerged, which is practically devoid of classical adhesion complex components but is instead highly enriched in proteins that promote **endocytosis** [7]. Indeed, it is increasingly recognized that there is extensive crosstalk between integrin-based adhesion sites, microtubules, and intracellular transport pathways, both to and from the cell surface [5–8]. While integrins themselves are subject to tight regulation by the trafficking machinery [9–13], the mechanisms that control integrin trafficking are discussed in detail elsewhere [14,15]. Here, we will focus on the latest findings in the field, showing that crosstalk between integrin adhesion complexes and the trafficking machinery regulates the internalization and/or release of proteins, organelles, and microorganisms. Together, these studies illustrate nonconventional roles of integrins that are important for a wide variety of pathophysiological events, including **hemostasis**, immune responses, and tissue development and repair.

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Key Figure

Integrins Promote Exocytosis of Biosynthetic and Secretory Vesicles

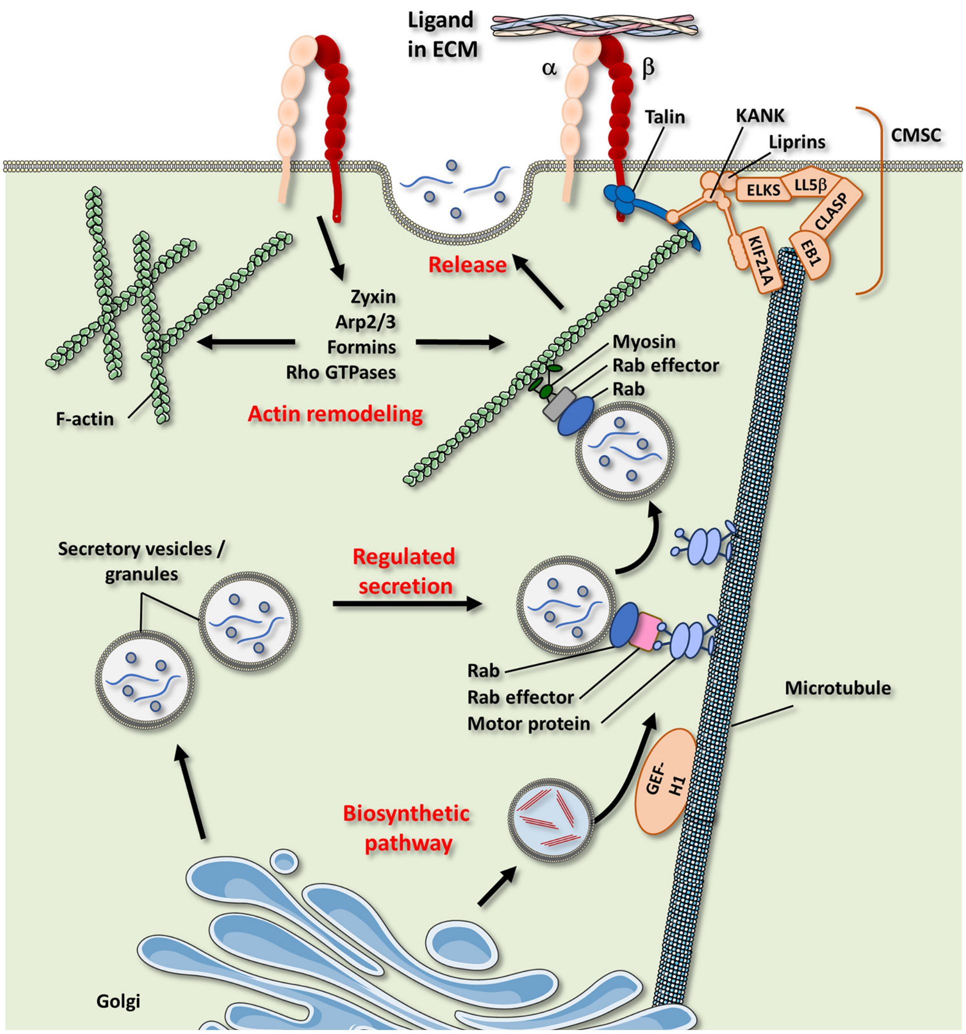

Figure 1. Integrins connect to the actin cytoskeleton via talin and remodel actin filaments via Rho GTPases, formins, and other proteins. Integrins also connect to microtubules via a complex of proteins, called the cortical microtubule stabilizing complex (CMSC) [22]. In this way, integrin adhesion sites are linked to the exocytic machinery, consisting of Rab GTPases, their effectors, and motor proteins. This machinery ensures the outward traffic of Golgi-derived vesicles carrying newly synthesized proteins (biosynthetic pathway), as well as secretory vesicles, which store proteins that are released in response to a specific cue (regulated secretion). Localized exocytosis of newly synthesized proteins occurs near integrin-controlled adhesion complexes [17,18,28] and is directed from the Golgi by guanine nucleotide exchange factor (GEF)-H1, an activator of RhoA that is associated with microtubules [29]. Abbreviations: CLASP, cytoplasmic linker associated protein; EB1, end-binding protein 1; ECM, extracellular matrix; ELKS, protein rich in amino acids E,L,K, and S; KANK, KN motif, and ankyrin repeat domain-containing; KIF21A, kinesin family member 21A.

Glossary

α-Granule: type of secretory granule found in platelets.

Anoikis: programmed cell death induced by loss of cell adhesion to the ECM.

Autophagosome: vesicular organelle involved in autophagy.

Autophagy: regulated degradation mechanism to remove dysfunctional or unnecessary components.

B cells: lymphocytes driving a humoral response by eliciting antibody formation.

Class switching: genetic recombination process in which B cells switch the production of a particular immunoglobulin isotype to another.

Clathrin-coated pits: plasma membrane invaginations important for clathrin-dependent endocytosis.

Complement: protein complex that enhances immune responses by antibodies and phagocytic cells.

Cortical microtubule stabilizing complex (CMSC): protein complex that captures and stabilizes microtubules at the plasma membrane.

Degranulation: release of factors from secretory vesicles called granules.

Dendritic cells (DCs): phagocytic white blood cells that activate T cells through antigen presentation.

Endocytosis: uptake of extracellular or plasma membrane components.

Exocytosis: release of molecules from a cell.

Exosome: extracellular vesicle released by exocytosis from multivesicular bodies.

Extracellular matrix (ECM): meshwork of proteins surrounding cells in tissues.

Extracellular vesicle (EV): cell-derived vesicle involved in cell–cell communication.

Flat clathrin lattice (FCL): specialized adhesion structure formed by integrin αvβ5 that is enriched in clathrin and other components of the endocytic machinery.

Focal adhesion (FA): adhesion structure where integrins connect the ECM to the cytoskeleton.

Germinal center (GC): sites in secondary lymphoid organs where B cell maturation and antibody affinity regulation occur.

Hemostasis: process that stops blood loss from damaged vessel.

Immunological synapse: adhesion structure formed by integrins to establish contact between a leukocyte and a target cell.

Connections of Integrin Adhesion Complexes to Microtubules and the Trafficking Machinery

Intracellular trafficking of organelles relies on **Rab GTPases**, which in their active, GTP-bound state are recruited to intracellular membranes and promote vesicle transport, fusion, and fission. Rabs enable the movement of organelles via effector proteins that can simultaneously bind activated Rabs and motor proteins such as myosins (which move along actin) or kinesins/dyneins (which move along microtubules) (Figure 1) [16]. Microtubules growing toward the cell surface are captured and stabilized by integrin adhesion complexes, via proteins that bind their growing or 'plus' ends, including cytoplasmic linker-associated proteins (CLASPs) [17–20]. These interact at the plasma membrane with a protein complex containing LL5 family (also called Pleckstrin homology-like *domain family* B) of proteins, which bind phospholipids in the plasma membrane, and ELKS (ERC1/Rab6 interacting/CAST family member 1/RAB6IP2) (Figure 1) [5,17–19,21,22]. The latter is an effector protein for the small GTPase Rab6, which regulates bidirectional traffic between the Golgi network and the cell surface [23]. The entire complex is defined as the **cortical microtubule stabilizing complex (CMSC)**, and consists furthermore of adapter proteins called liprins and the recently identified KN motif and ANKyrin repeat domain-containing (KANK) proteins [21,22,24–26]. KANKs associate with liprins and the kinesin KIF21A, while their N terminal domains bind directly to talin, and thereby link microtubules to integrins (Figure 1) [22,25,26]. Since microtubules provide polarized tracks for long-range vesicular transport, integrins thus target the trafficking machinery to discrete membrane domains. In addition, many exocytic vesicles use actin filaments for movement, which is also indirectly affected by adhesion complexes, either because integrins are linked to actin filaments, or because they control local actin remodeling via proteins such as zyxin, formins, Rho GTPases, and the Arp2/3 complex (Figure 1) [2]. Finally, the cytoplasmic tails of some integrin β-subunits can also bind directly to the motor protein Myosin-10, which regulates adhesion and filopodia formation [27]. Thus, several structural and functional interactions exist that link integrin adhesion complexes, cytoskeletal elements, and the intracellular trafficking machinery.

Integrin Adhesion Complexes Regulate Exocytosis of Biosynthetic and Secretory Vesicles

Golgi-derived vesicles in the biosynthetic pathway are transported along microtubules to the cell surface under the control of Rab6 or Rab8 in a myosin-II-dependent manner, where they dock and fuse with the plasma membrane preferentially in the vicinity of adhesion complexes (Figure 1) [17,19,23]. This has been observed in epithelial cells such as keratinocytes, as well as in cancer cells, and requires the capture of microtubules through CLASP-LL5 interactions, while ELKS regulates vesicle docking [17–19,21,23]. Using an assay to induce synchronized release of proteins from the endoplasmic reticulum into the biosynthetic pathway, it was recently revealed that ECM proteins, but also a variety of other cargos, are exocytosed near adhesion complexes [28,29]. This is an intriguing finding since it suggests that membrane domains near adhesions are particularly permissive for exocytosis in general. Furthermore, localized delivery is already directed from the Golgi, in a manner dependent on Rho and guanine nucleotide exchange factor (GEF)-H1 (Figure 1), an activator of Rho GTPases associated with microtubules [29].

In addition to regulating outward transport along the biosynthetic pathway, integrins are also emerging as regulators of secretory vesicle exocytosis (Figure 1). In the pancreatic islets of Langerhans, β-cells make and secrete insulin into the vasculature. The β-cells are polarized by contacts with the basement membrane that surrounds capillaries, where integrins assemble adhesion complexes enriched in liprins and ELKS [30]. These integrins direct insulin-containing granules to the cell surface, which is disrupted by blockade of integrin–ligand binding, or by pharmacological inhibition of focal adhesion kinase (FAK), an important signaling component in

Interferons (IFNs): cytokines secreted in response to infection.
Macrophages: phagocytic white blood cells that destroy pathogens and apoptotic cells.
Microtubules: cytoskeletal filaments that transport vesicles to and from the cell periphery.
Microvesicle: extracellular vesicle derived from the cell surface by budding.
Natural killer cell: innate immune cells that clear viral infections or cancer cells.
Opsonization: coating of a particle to facilitate phagocytosis.
Phagocytosis: engulfment of large particles, such as microbes or apoptotic cells, by specialized cells.
Platelets: cell fragments in the circulation, required for blood clotting.
Rab GTPase: small protein residing on intracellular membranes that mediates intracellular transport.
Receptor tyrosine kinase (RTK): transmembrane receptor with intrinsic tyrosine kinase activity that is activated by growth factor binding.
Reticular adhesion: adhesion complex similar to flat clathrin lattice.
RGD: amino acid motif present in many integrin ligands, including ECM proteins.
T cells: lymphocytes that, when activated, recognize and kill virus-infected and cancerous cells (CD8), or help other immune cells (CD4).
Toll-like receptors: transmembrane immune receptors that detect particular molecules derived from pathogens.
von Willebrand Factor (vWF): large multimeric adhesive protein required for hemostasis, released from endothelial cells.
Weibel-Palade body (WPB): secretory vesicles in endothelial cells that store and release vWF.

FAs [30]. Indeed, the targeted deletion of the gene encoding FAK in β-cells impairs insulin secretion in mice due to impaired granule targeting to the plasma membrane, while glucose sensing and response to glucose are normal [31]. By contrast, deletion of the gene encoding β1 in these cells does not disrupt insulin secretion in mice, which may indicate functional redundancy among integrins in regulating this process [32].

In endothelial cells, several FA proteins were found to promote the secretion of **von Willebrand factor (vWF)** from secretory organelles called **Weibel-Palade bodies (WPBs)**. The strongest effect was observed for zyxin, which is an actin-binding protein important for the connection of FAs to actin filaments. Zyxin is required for actin remodeling around sites of WPB exocytosis. Since vWF secretion from WPBs is required for the trapping of **platelets** and subsequent platelet aggregation, mice that do not express zyxin suffer from prolonged bleeding times and impaired thrombus formation [33]. Intriguingly, it remains to be determined which subcellular zyxin pool is responsible for the observed effects, since WPB release of vWF occurs primarily at the apical surface facing the vascular lumen, while FAs are mostly assembled on the basal surface where integrins bind the basement membrane.

Integrin Adhesion Complexes Regulate Platelet and Leukocyte Degranulation

Integrins are essential for hemostasis and immunity, because they regulate platelet aggregation, leukocyte adhesion and migration, and transforming growth factor-β activation [34,35]. In addition, it is becoming clear that integrins also control vesicular trafficking in platelets and leukocytes at several levels. In activated lymphocytes, the immunological synapse is a prime example of an integrin-formed structure that is also a site of heavy intracellular traffic [36]. In cytotoxic **T cells**, the recognition of cognate antigen on an adjacent target cell will induce microtubule anchoring at the peripheral supramolecular activation cluster; a ring surrounding the clustered T cell receptors that consists of abundant αLβ2 ligated to intercellular adhesion molecule (ICAM) on the adjacent target cell [37]. Driven by T cell receptor signaling, this adhesion complex enables the polarized **degranulation** of lytic granules into the immunological synapse (Figure 2A) [38]. Similarly, αLβ2–ligand interactions also cooperate with other receptors at the cell surface to induce granule convergence in **natural killer cells** (i.e., the orientation of granules at the microtubule organizing center and their subsequent directed exocytosis), which enhances specific targeting and reduces a-specific 'bystander' killing [39]. Integrin-dependent granule convergence is achieved by a number of regulatory protein networks downstream of αLβ2, including common components of integrin-based adhesions such as integrin-linked kinase, Pyk2, and paxillin [40]. Furthermore, the binding of integrin αEβ7 on CD8 T cells to E-cadherin on tumor cells mediates lytic granule polarization and subsequent exocytosis [41]. Because lytic granule release requires interactions with cortical actin filaments, proteins that are important for local actin remodeling, such as zyxin, may also be involved here, as described earlier for endothelial cells.

Integrin function in platelets is also intimately linked to the degranulation machinery. In isolated human platelets *in vitro*, **α-granule** exocytosis occurs primarily at sites where integrins promote platelet spreading, through activation of Rac-dependent actin polymerization [42]. Rac is also required for degranulation, and the release of fibronectin and fibrinogen from α-granules stimulates further integrin ligation and platelet spreading (Figure 2B). Thus, a feedforward loop exists between integrins, Rac activation, and degranulation [43]. Importantly, granule secretion and integrin αIIbβ3-dependent platelet aggregation *in vivo* are still supported by β1 integrin amounts as low as 3% of the normal expression levels, whereas this is not sufficient for platelet adhesion. This suggests that β1 integrins regulate hemostasis predominantly by promoting granule release, rather than by platelet adhesion [43]. Integrin-mediated Rac activation and platelet spreading, as well as RhoA-dependent clot retraction, also require Vps33B, a component of sorting/tethering

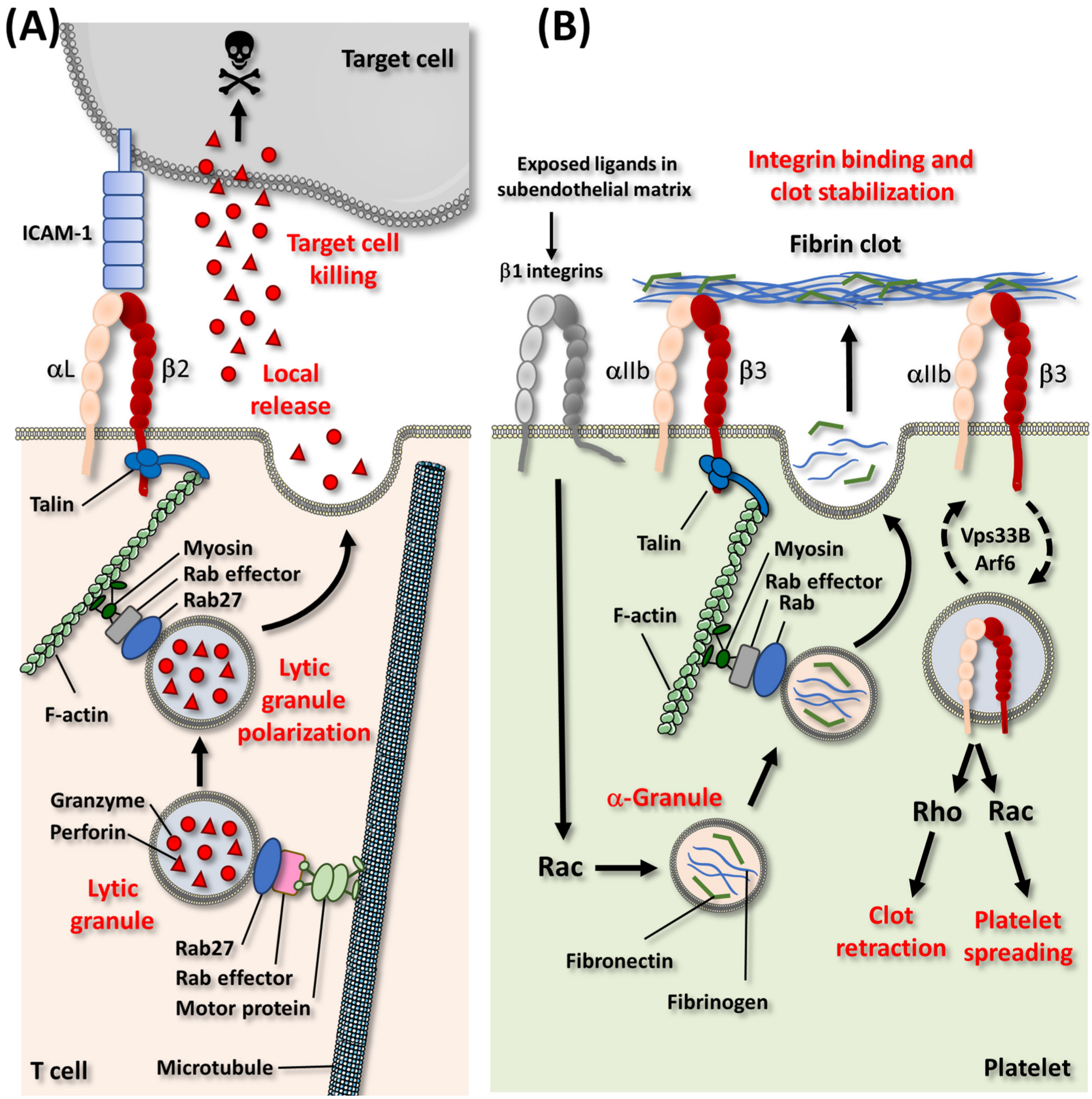

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Figure 2. Integrins Control Leukocyte and Platelet Degranulation, Important for Immunity and Hemostasis. (A) Upon antigen recognition on target cells by cytotoxic T cells, a tight interaction is established through αLβ2 and intercellular adhesion molecule (ICAM), which enables local release of lytic granules to kill the target cell, thereby preventing collateral damage to other cells [37–39]. Integrin-dependent granule convergence is achieved by a number of regulatory protein networks downstream of αLβ2 [40] and directed granule trafficking through Rab27. (B) In platelets, activation of the integrin αIIbβ3 is essential for platelet spreading and aggregation, as well as clot formation and retraction. Ligation and signaling from β1 integrins are required for the release of fibronectin and fibrinogen from platelet α-granules, which stimulates further platelet spreading and aggregation [42]. Moreover, Rho-mediated clot-retraction and Rac-mediated platelet spreading are dependent on αIIbβ3 endocytic trafficking, which is regulated by ADP-ribosylation factor 6 (Arf6) and vacuolar protein sorting-associated protein 33B (Vps33B) [44,46].

complexes on vesicular compartments [44]. Vps33B promotes the biogenesis of α-granules, as well as their exocytosis, and patients with mutations in Vps33B develop a multiorgan disorder with bleedings [45]. Intriguingly, Vps33B binds directly to β1 and β3 integrin cytoplasmic tails at a site partially overlapping with that of talin and was previously shown to localize to recycling vesicles, which transport internalized integrins [13,44]. Thus, Vps33B links integrin function and trafficking to platelet degranulation. Another trafficking protein, the GTPase Arf6, was also shown to enhance platelet spreading [46]. Because Arf6 is required for fibrinogen uptake by αIIbβ3, it may link integrin turnover to platelet function.

Integrin Crosstalk with the Endocytic Machinery Regulates Adhesion Turnover, Mechanotransduction, and Phagocytosis

Integrins also have extensive crosstalk with the endocytic machinery. Microtubules deliver endocytic machinery to FAs and trigger their disassembly by internalizing FA components via clathrin-mediated endocytosis [6,8,47,48]. Clathrin adapter proteins, such as AP-2, ARH, Dab2, and Numb, bind directly to the cytoplasmic tails of integrin α- or β-subunits and recruit them into **clathrin-coated pits**, which are subsequently internalized and delivered to early endosomes (Figure 3) [12,14,49]. Indeed, FA disassembly is blocked by disruption of microtubule

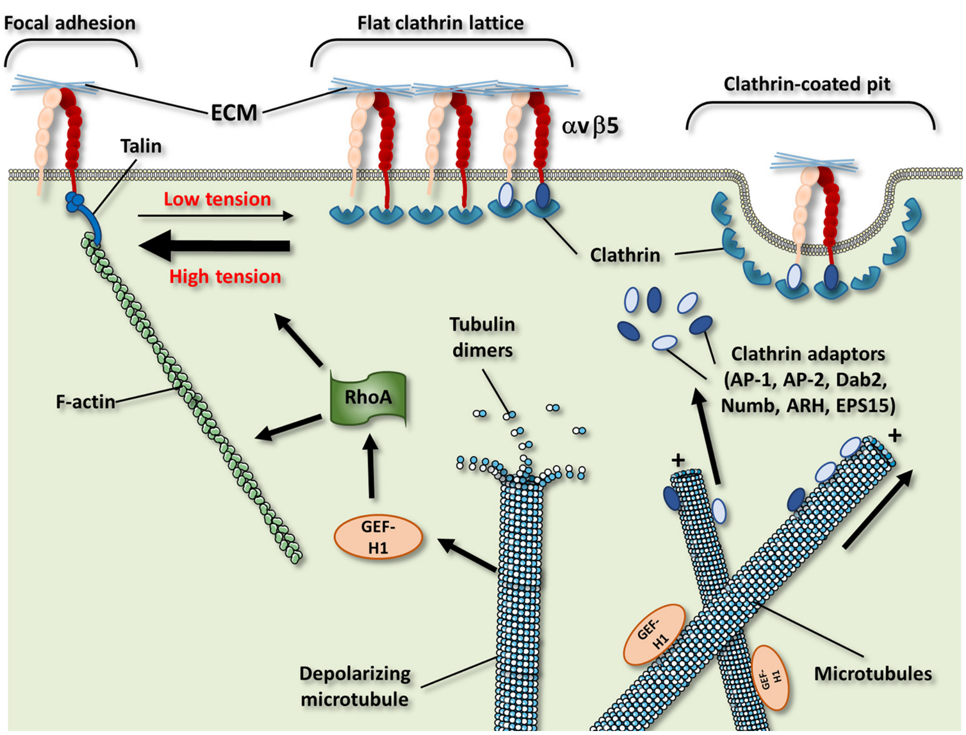


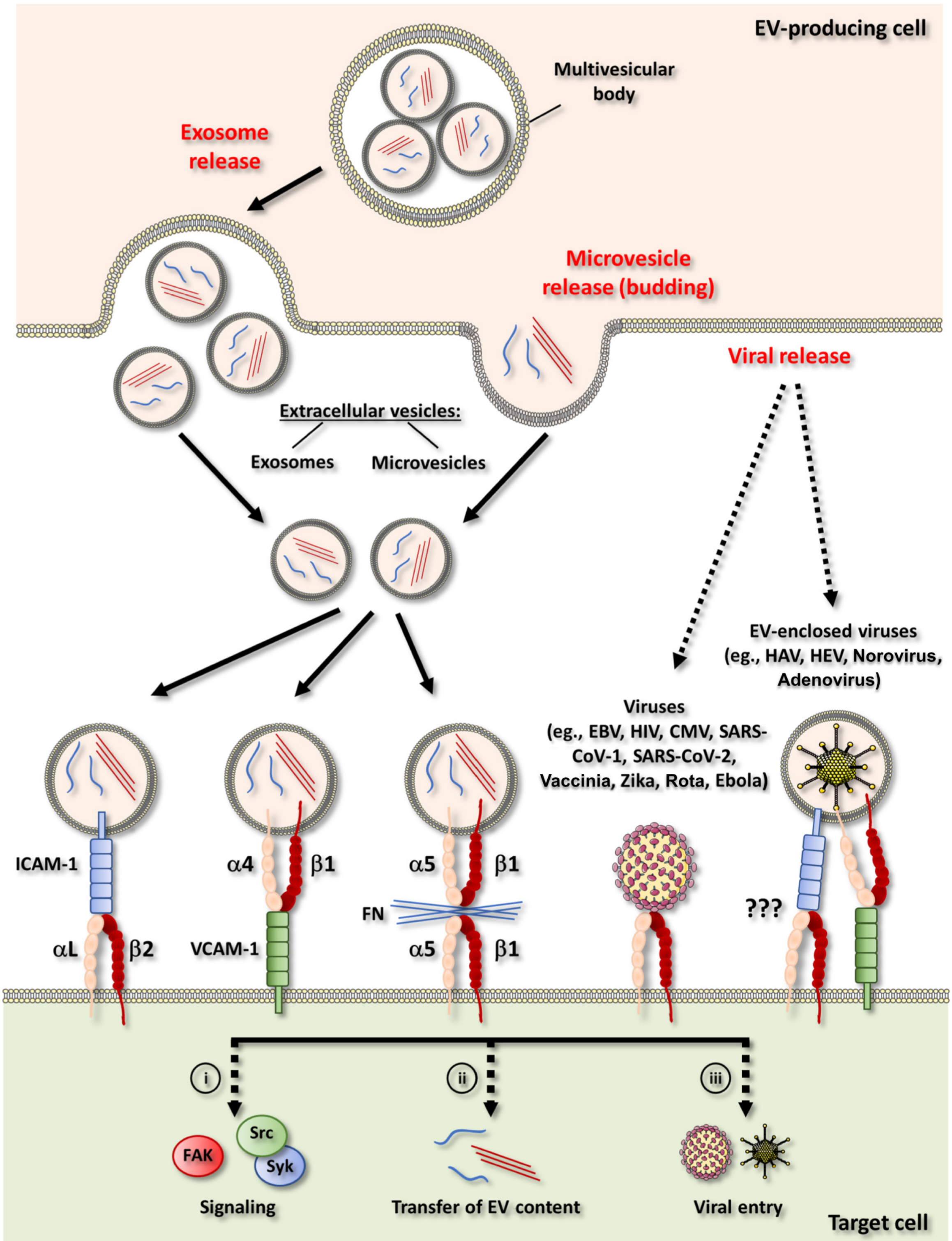
Figure 3. Integrin Crosstalk with the Clathrin Machinery Regulates Adhesion Turnover, Mechanotransduction, and Endocytosis. Microtubules deliver clathrin adaptors that trigger disassembly of focal adhesions (FAs) and recruit the integrins and their ligands into clathrin-coated pits [47,48]. Binding of clathrin adaptors to integrins is also involved in the formation of the more static flat clathrin lattices (FCLs), which develop when αvβ5 forms stable interactions with immobilized vitronectin; this prevents the formation of pits and thereby 'frustrates' the endocytic process [52,55,59]. These structures depend on high rigidity of extracellular matrix (ECM) ligands, but are associated with low intracellular tension, as they do not depend on myosin II-activity [60]. Yet, increases in cytoskeletal tension, for example, by guanine nucleotide exchange factor (GEF)-H1/RhoA-mediated actomyosin contractility in response to microtubule depolymerization, stimulate FA assembly and integrin translocation from the FCLs to FAs [59,65]. Abbreviations: AP, adaptor protein; ARH, autosomal recessive hypercholesterolemia; Dab2, Disabled-2; EPS15, epidermal growth factor receptor pathway substrate 15.

polymerization, in part because endocytic machinery is no longer delivered to FAs, but also because GEF-H1 is released from microtubules under these conditions, thus triggering a local burst in myosin-IIA filament assembly via the RhoA-Rho kinase pathway [26,50].

In addition to clathrin-coated pits, clathrin can also assemble very large, sheet-like structures named clathrin-coated plaques, clathrin sheets, or FCLs [51–54]. FCLs are highly enriched in endocytic proteins, but in contrast to pits, they are static and long-lived structures that persist throughout the cell cycle, even during mitosis [55]. Most endocytic proteins in FCLs distribute to the periphery of these structures, which is indeed also where budding pits are observed (Figure 3) [56]. This is consistent with the observation that clathrin coats first grow flat, but that bending begins upon a change in the ratio between clathrin and the adapter AP-2 [57,58]. Hence, the center of the FCL has very low endocytic activity and its assembly is considered to result from 'frustrated endocytosis' [52]. It is now increasingly recognized that this 'frustration' is the result of tight adhesion of FCLs to the substrate by integrin αvβ5 (Figure 3) [7,55,59]. Whereas αvβ5 can efficiently uptake several ligands such as vitronectin, interaction of this integrin with immobilized vitronectin prevents the formation of pits, but stimulates plaque formation [60]. Similarly, β1 integrins have been shown to promote the formation of tubular clathrin lattices along collagen fibers, which regulate cell adhesion in an endocytosis-independent manner [61]. Therefore, FCLs are now increasingly recognized as a novel type of adhesion complex (and have also been named '**reticular adhesions**'), although they lack classical adhesion components and have low amounts of talin [7,59]. Furthermore, FCLs are not linked to actin stress fibers like FAs, but they are associated with branched cortical actin, generated by the Arp2/3 complex [51,62]. In turn, cortical actin recruits the intermediate filament system to these structures [63]. FCL assembly requires αvβ5-vitronectin interaction and recruitment of clathrin adaptors such as ARH, Numb, and EPS15/EPS15L1 to the β5 cytoplasmic tail [59]. Integrin cytoplasmic tails can induce profound differences in the behavior of distinct integrins, even when they bind the same ligand [64], which is most likely due to differences in the relative affinities for specific proteins. The cytotail of αvβ5 contains an insert of eight amino acids, as compared with integrin β1 or β3 tails [34], and may have an exceptionally high affinity for clathrin adaptors. Consistent with this idea is the observation that swapping the β5 tail with that of β1 or β3 induces a redistribution to FAs [59]. Although FCL assembly increases with high substrate rigidity, their assembly is independent of myosin-II activity [60]. In fact, high intracellular tension generated by actomyosin contractility triggers translocation of αvβ5 to FAs, while low tension promotes its localization to FCLs (Figure 3) [59,65]. Conversely, recruitment of Dab2 and Numb to ligated αvβ3, as well as a loss of talin from these complexes, is promoted by the absence of physical forces [66]. Thus, modulation of cellular tension affects selective integrin recruitment to adhesion complexes. In addition, FCLs also regulate signaling and cell proliferation [51,60]. So far, FCLs have only been observed in muscle *in vivo*, but they are likely important in other cell types as well [62,63]. For example, **macrophages** are specialized for the uptake of large objects such as microorganisms or apoptotic cells by **phagocytosis**, which is dependent on integrin αvβ5 [67,68]. Indeed, deletion of the gene encoding β5 in hematopoietic cells in mice impairs tissue repair by intestinal macrophages and increases susceptibility to chemical colitis [68]. Furthermore, retinal pigment epithelial cells rely on αvβ5 for the phagocytosis of retinal debris such as spent photoreceptor outer segment fragments, which is critical for vision. In the absence of αvβ5, mice fail to clear this debris and develop age-related blindness [69]. It is conceivable that FCLs affect phagocytosis in specialized cell types, which will require further investigation.

Integrins Control Uptake of Extracellular Vesicles and Viruses

Another emerging role for integrins in vesicle traffic relates to the binding and uptake of **extracellular vesicles (EVs)**. These are cell-derived, 50–1000 nm sized vesicles of different subcellular origin, including **exosomes**, which originate from late endosomal compartments (multivesicular bodies), and **microvesicles**, which bud from the plasma membrane (Figure 4) [70]. Accumulating evidence



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implicates EVs in a wide range of pathophysiological processes, including tissue regeneration, cancer, and cardiovascular disease. Virtually every cell type can produce EVs, which enables them to dispose of intracellular content or to communicate with other cells. The latter is mediated either by EV-induced signaling at the target cell membrane, or by transfer of their molecular cargo, such as small RNAs, proteins, or lipids, to target cells [70]. Integrins play an important role herein, as EVs derived from many different cell types contain integrin ligands, including ICAM-1 and/or vascular cell-adhesion molecule-1, which enables them to bind β2 or β1 integrins on the target cell (Figure 4). For example, EVs produced by **dendritic cells (DCs)** can bind to activated T cells and to DCs through αLβ2 [71,72]. Conversely, various β1 and β2 integrins have also been detected on EVs, allowing these vesicles to bind to target cells and/or ECM components [73–75]. Interestingly, integrins on EVs can even mediate tumor metastasis to a particular tissue: αvβ5 mediates tumor-EV binding to Kupffer cells, which facilitates liver metastasis, whereas α6β4 facilitates binding to lung fibroblasts and thereby promotes metastasis to the lungs [76]. Recent data indicate that integrins in cancer cell EVs may also regulate tumor-induced angiogenesis. Integrin αvβ6-containing EVs released by prostate cancer cells mediate αvβ6 transfer to microvascular endothelial cells and promote endothelial cell motility and tube formation, in contrast to EVs isolated from β6 negative cancer cells [77]. Finally, integrins can regulate the sorting of fibronectin into cancer cell EVs, as well as the contents and antibacterial activity of neutrophil EVs, although how integrins crosstalk with the sorting machinery remains to be established [75,78].

In addition to EVs, a variety of pathogens, including bacteria and viruses, can also use integrins such as αvβ3 and αvβ5 for cell attachment, which is of interest for possible therapeutic intervention (Figure 4) [79,80]. For example, Zika virus uses αvβ5 to enter target cells and blocking this integrin reduces viral infection and alleviates virus-induced pathology [81]. Moreover, coronaviruses may also use integrins to infect cells. Accessory protein-7a of the severe acute respiratory syndrome (SARS)-coronavirus SARS-CoV-1, interacts directly with αLβ2 [82]. In addition, the spike protein of SARS-CoV-2, which causes coronavirus disease 2019 (COVID-19), contains an integrin-binding **RGD** motif that is absent from other coronaviruses [83].

Various naked viruses, such as *Picornaviridae* and *Hepeviridae*, can escape infected cells already in the prelytic phase of infection, via packaging and release within EVs (Figure 4) [84,85]. This stealth mode prevents viruses from being neutralized by antibodies, but these virions also become dependent on EV-targeting pathways to infect new cells, as they are cloaked by a host-derived membrane. To what extent these EV-enclosed virions express the same adhesion molecules as normal EVs remains to be investigated, though it is likely that integrins are also involved in this novel mechanism of viral dissemination. Indeed, both naked and EV-enclosed virions of hepatitis A virus are dependent on β1 integrins for viral entry, although they interact with distinct integrin domains [86]. It will be important to determine how viruses and EVs make use of overlapping mechanisms for target cell binding and cargo delivery and to what extent integrins play a role.

Figure 4. Integrins Control Binding and Uptake of Extracellular Vesicles and Viruses. Extracellular vesicles (EVs) are either derived from multivesicular bodies (exosomes), or bud from the plasma membrane (microvesicles). Integrins and/or integrin ligands on the surface of EVs can mediate EV binding to target cells. Following binding, EVs can induce signaling into the target cell (i) and/or transfer their content into the cell (ii). Enveloped viruses can use envelope proteins to bind integrins on target cells prior to viral entry. Various naked viruses can be released by infected cells at the prelytic stage via packaging into host EVs, which may allow integrin-mediated entry of target cells (iii). Abbreviations: CMV, Cytomegalovirus; CoV, coronavirus; EBV, Epstein-Barr virus; FAK, focal adhesion kinase; FN, fibronectin; HAV, hepatitis A virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; ICAM, intercellular adhesion molecule; SARS, severe acute respiratory syndrome; VCAM-1, vascular cell adhesion molecule-1.

Integrin-Dependent Vesicular Trafficking Regulates Receptor Signaling and Autophagy

In addition to the multiple roles of integrin adhesion complexes in vesicular trafficking, integrins also have more direct roles in this process. Some integrins are cotrafficked through the endosomal system with **receptor tyrosine kinases (RTKs)**, such as epidermal growth factor receptor and vascular endothelial growth factor receptor-2 (Figure 5) [87,88]. The relationship between the involved RTKs and integrins is reciprocal, as the RTKs affect integrin traffic and vice versa. This is controlled by growth factor stimulation, as well as by proteins that associate with the cytoplasmic tails of these receptors, including kinases and Rab-coupling protein [87,89,90]. While it has long been known that integrins signal in a synergistic manner with growth factors to regulate cell survival, proliferation, and migration, it is now apparent that both integrin and RTK signaling does not occur exclusively from the plasma membrane, but continues from endosomal compartments after internalization (Figure 5). In fact, endocytosis of ligand-occupied integrins is required to achieve optimal signaling toward FAK, as well as to the AKT and extracellular signal-regulated kinase (ERK) pathways [91]. Endosomal integrin signaling can potentiate RTK signaling from these compartments and contributes to anchorage-independent growth and **anoikis** resistance in cancer cells [91,92]. Integrin trafficking may also regulate proliferation at the level of nutrient sensing, as it has been shown that the delivery of internalized, ligand-engaged α5β1 integrins to late endosomes/lysosomes regulates the recruitment and activation of mammalian target of rapamycin, a key regulator of cell growth in response to nutrients [93].

It is also becoming clear that integrins associate with pathways that regulate **autophagy**, which is responsible for the recycling of nutrients during starvation as well as for antigen presentation and the digestion of microbes. Autophagy involves the formation of intracellular compartments, including **autophagosomes**, that are coated with microtubule-associated protein 1A/1B light chains 3B (LC3) and fuse with lysosomes to degrade their contents [94]. Intriguingly, internalized β1 integrins cotraffic with activated c-Met toward LC3-containing vesicles in cancer cells, where they sustain c-Met signaling [92]. Furthermore, αv integrins, most importantly αvβ3, can stimulate the recruitment of **Toll-like receptor (TLR)** 9 into LC3-positive compartments in **B cells** (Figure 5), and deletion of the genes encoding either αv or β3 results in enhanced and prolonged TLR signaling and increased B cell activation [95]. It remains to be determined how αvβ3 exerts these effects, but because αvβ3 can directly interact with TLRs, the integrin–TLR complex possibly cotraffics through the endosomal system [96]. Furthermore, it is well established that the cytoplasmic tail of β3 is particularly effective in recruiting Src kinase and spleen tyrosine kinase (Syk), which can negatively regulate TLR signaling (Figure 5). While TLR signaling toward NF-κB and **interferon (IFN)**-regulatory factor pathways promotes B cell function in **germinal centers (GCs)**, it needs to be tightly regulated to prevent the generation of high-affinity autoantibodies [97]. Indeed, increased TLR9 signaling induced by αvβ3 deletion causes expansion of GC, memory, and plasma cells and increases **class switching** and somatic hypermutation. Together, these events strongly augment the generation of high-affinity antibodies, both to foreign and 'self' antigens associated with TLR ligands [98]. It is conceivable that αvβ3 functions as a 'sensor' that regulates TLR signaling, stimulating necessary responses against pathogens but preventing excessive responses leading to autoimmunity. This hypothesis fits well with studies showing that αvβ3 functions as a coreceptor for the phagocytosis of viruses and microbes and enhances TLR-mediated innate immune responses induced by herpes simplex virus or bacterial ligands [99,100]. Moreover, several observations suggest that αMβ2 may similarly control the balance in TLR signaling. First, αMβ2 stimulates the uptake of *Listeria monocytogenes* and other (**complement**-**opsonized**) bacteria into LC3-positive compartments [101,102], and negatively regulates TLR-dependent proinflammatory signaling and IFN

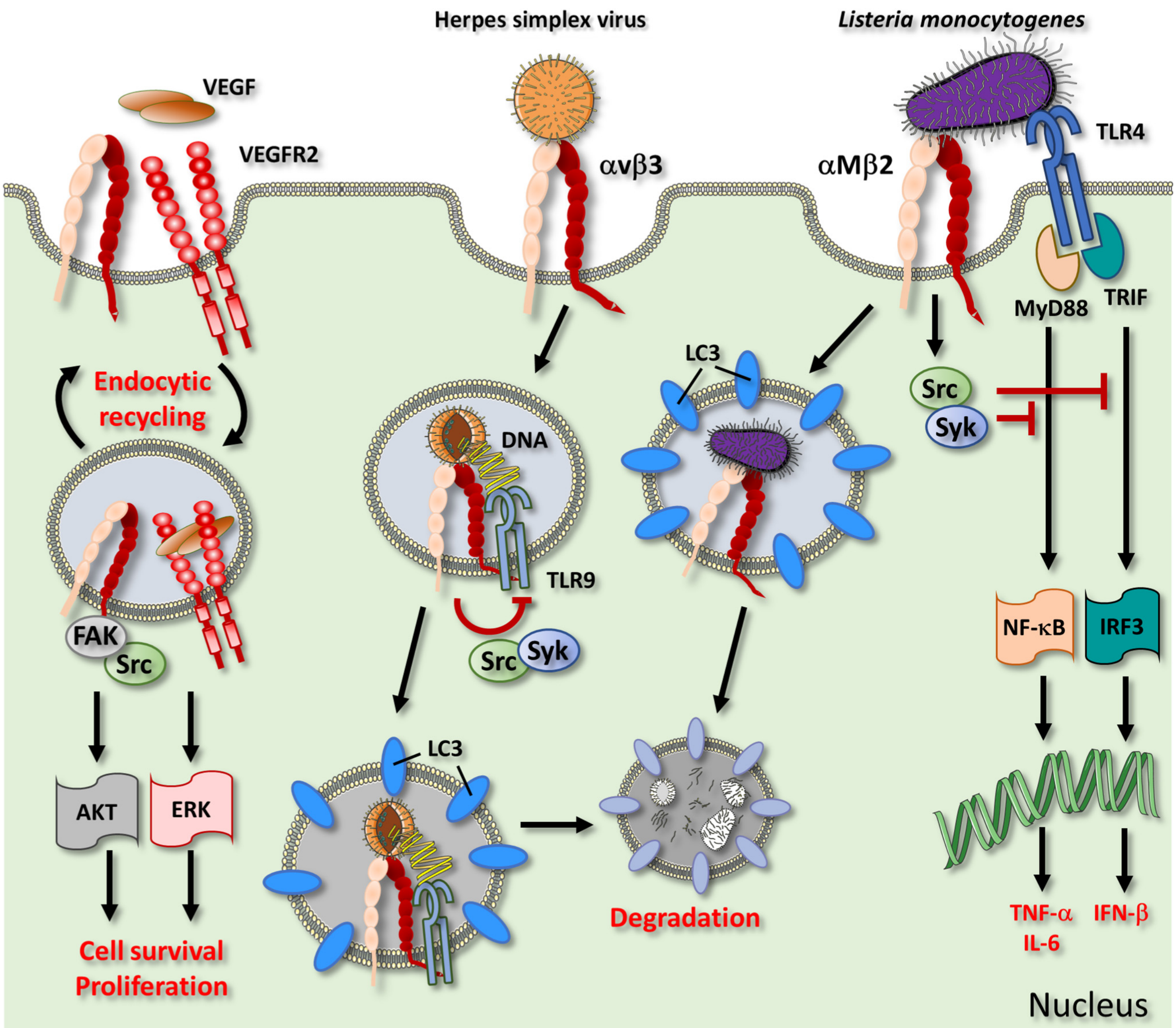

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Figure 5. Integrin Regulation of Vesicular Trafficking and Autophagy Pathways Controls Receptor Signaling. Integrins regulate endocytic recycling of receptor tyrosine kinases, such as vascular endothelial growth factor receptor 2 (VEGFR2), under the control of growth factors and proteins that associate with their cytoplasmic tails [87,89,90]. Moreover, internalized integrins cosignal with these receptors from endosomes toward the extracellular signal-regulated kinase (ERK) and AKT pathways, to synergistically drive cell proliferation and survival [91,92]. Some integrins mediate phagocytosis of (complement-opsonized) pathogens, such as herpes simplex virus and *Listeria monocytogenes* [99,100]. Following internalization, these integrins can induce the recruitment of microtubule-associated protein 1A/1B light chains 3B (LC3) to form autophagosomes, which fuse with lysosomes to degrade the pathogens [94,95]. Integrin αvβ3 can directly interact with certain Toll-like receptors (TLRs) and limit their signaling, possibly through recruitment of Src and Syk, thereby decreasing cellular activation [95]. Moreover, αMβ2 signaling through Src and Syk can also inhibit TLR signaling toward NF-κB and interferon-regulatory factor 3 pathways [101–105]. Several integrins may therefore function as a 'sensor' that regulates TLR signaling by stimulating necessary responses against pathogens but preventing excessive responses leading to autoimmunity. Abbreviations: FAK, focal adhesion kinase; IL, interleukin; IFN, interferon; IRF, interferon-regulatory factor; MyD88; myeloid differentiation primary response 88; NF-κB, nuclear factor-κ of activated B cells; Syk, spleen tyrosine kinase; TNF, tumor necrosis factor; TRIF, TIR-domain-containing adapter-inducing interferon-β; VEGF, vascular endothelial growth factor.

production, in a manner involving Src and Syk [103] (Figure 5). Second, genetic variations in the αM-subunit are strongly associated with systemic lupus erythematosus and it is now clear that these mutations not only lead to dysfunctional integrins that cannot promote cell adhesion or phagocytosis, but also to enhanced proinflammatory signaling [104]. Finally, pharmacological activation of αMβ2 suppressed TLR-dependent inflammation and autoimmunity in a mouse model for lupus [105]. Thus, it is now clear that integrins can control signaling pathways initiated by a variety of receptors, by directing phagocytosis, autophagy, and receptor trafficking.

Concluding Remarks

Integrins have now firmly emerged as crucial regulators of vesicular traffic in a wide range of cell types. Not only do they target the biosynthetic machinery toward the plasma membrane, but they are also involved in a variety of endocytic, phagocytic, and secretory events that together regulate many aspects of human health and disease. Much remains to be learned about the involved machinery and the signals that drive integrin-dependent vesicular transport (see Outstanding Questions). Further research is required to determine the regulation and *in vivo* function of FCLs, the function of integrin–autophagy crosstalk in immune responses, and the role of integrins in the transfer of EVs and viruses. Finally, it will be important to explore if the described mechanisms can be employed therapeutically.

Outstanding Questions

What are the signals driving vesicle exocytosis near integrin adhesion complexes?

Do FCLs regulate integrin signaling and/or trafficking?

What is the physiological role of FCLs/reticular adhesions?

How are integrins and their ligands sorted into EVs?

Can cell-to-cell spreading of (EV-enclosed) viruses be inhibited by targeting integrins?

How do αv integrins affect TLR sorting and signaling and can this be exploited therapeutically to modulate immune responses?

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