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REVIEW

Integrins: Structure and Signaling

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Abstract—Integrins are cell surface transmembrane glycoproteins that function as adhesion receptors in cell–extracellular matrix interactions and link the matrix proteins to the cytoskeleton. The family of human integrins comprises 24 members, each of which is a heterodimer consisting of 1 of 18 α- and 1 of 8 β-subunits. Integrins play an important role in the cytoskeleton organization and in transduction of intracellular signals, regulating various processes such as proliferation, differentiation, apoptosis, and cell migration. This review summarizes current views on the structure of integrins, integrin associated proteins, and biochemical mechanisms underlying their signaling functions.

Key words: integrins, extracellular matrix, cytoskeleton, signaling

Integrins are a large family of surface receptors that are localized on plasma membrane and share common features in molecular structure and functions. Integrins are involved in cell interactions with extracellular matrix glycoproteins (collagen, fibronectin, laminin, etc.); some integrins are also involved in intercellular interactions [1-3]. After identification of integrins as an independent class of cell receptors, their role in adhesion (i.e., cell adhesion to the extracellular matrix) was considered as the principal or even the only function. However, even before the discovery of integrins it was known for a long time that matrix serves not only as a backbone for spatial organization of a tissue; it can influence cell behavior under various physiological and pathological conditions such as embryogenesis and differentiation, morphogenesis, tumor growth, apoptosis, etc. For many years mechanisms underlying these functions of extracellular matrix remained unclear.

The discovery of integrins and studies of their ligand specificity (with respect to extracellular matrix proteins) and interactions with intracellular macromolecules clarified many important aspects of this problem. These receptors were shown to connect extracellular matrix with intracellular structures and regulatory molecules controlling cell behavior [3-6]. The involvement of integrins in control of cell behavior is the second (signaling) function of integrins.

Recent studies have revealed that intracellular signaling realized by integrin-dependent mechanisms shares certain similarity with the signaling mechanisms in a cell in response to various changes of internal and/or external medium and which involve receptors other than integrins (e.g., during effects of hormones, growth factors, altered ionic environment, etc.) [2, 4, 7].

In this review we consider structural properties of various integrins, functions of domains of these receptors, and also the role of integrins in physiological reactions of cells and mechanisms underlying integrin-dependent signaling.

1. STRUCTURE OF INTEGRINS

All integrins share certain structural resemblance [1, 8]. Each receptor is a heterodimer consisting of one α- and one β-subunit. The dimer is stabilized by noncovalent bonds. Each subunit is an integral transmembrane polypeptide type I (i.e., NH_2-terminus of the polypeptide has extracellular localization whereas COOH-terminus is faced toward the cytoplasm). Each subunit contains three domains: glycosylated extracellular domain (which consists of more than 90% of the whole molecule), hydrophobic transmembrane domain (responsible for membrane anchoring) and endo- (or cytoplasmic) domain, localized in the cytoplasm. Figure 1 shows a schematic representation of the domain structure of the integrin subunits.

1.1. Structure of α-subunits. Sizes of α-subunits vary in the range 120-180 kD. At the NH_2-terminus all α-subunits have seven repeated homologous domains (I-VII), each of which consists of ~50 amino acid residues. Bivalent cation binding sites located in the central part of

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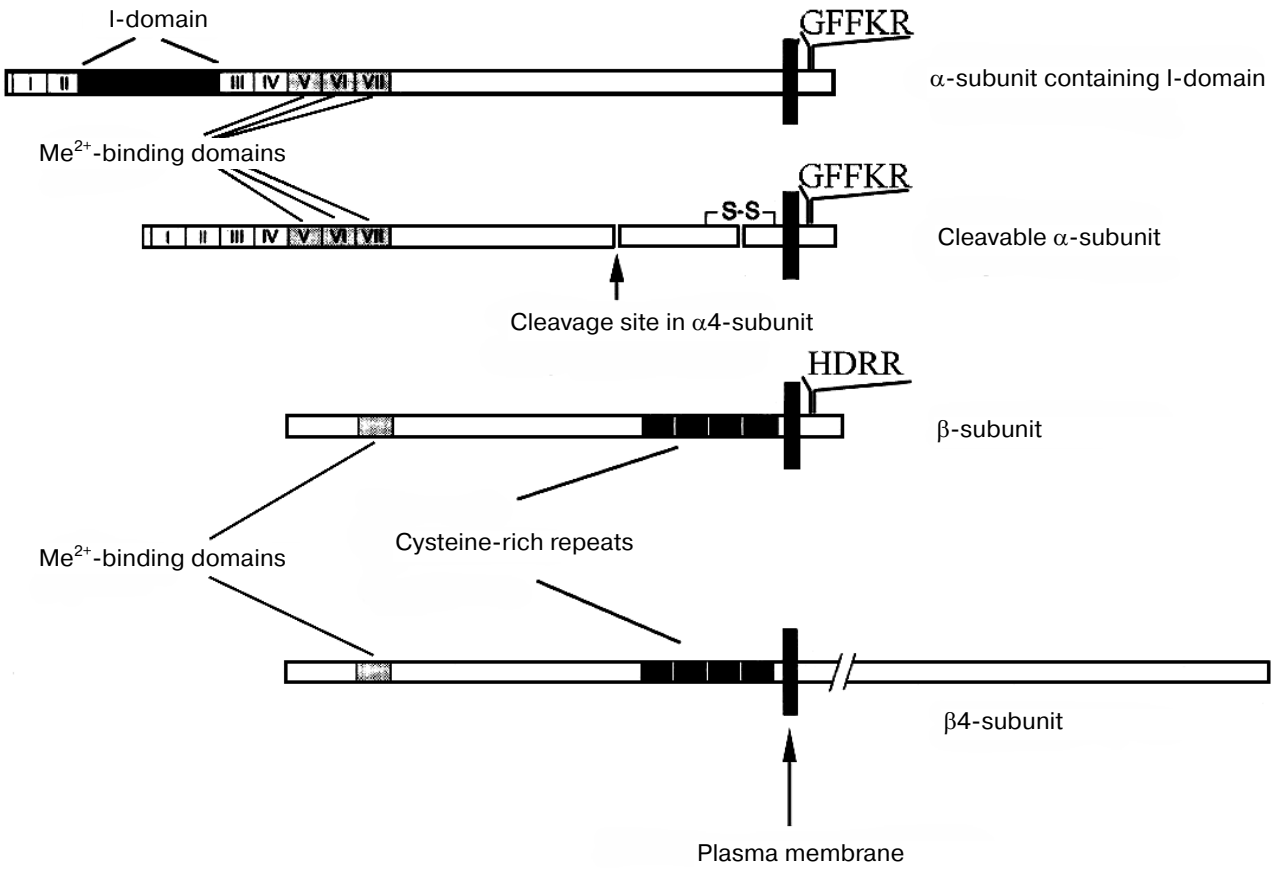

Fig. 1. Domain structure of integrin subunits [1]. Extracellular and cytoplasmic domains are shown on the left and the right sides of plasma membrane, respectively. Other explanations are in the text.

each repeat exhibit highest homology (Fig. 1). The secondary structure of these sites is very similar to Ca-binding motif (so-called EF-hand motif) found in some Ca-binding proteins [1, 9].

α-Subunits of collagen-specific integrins (α1β1, α2β1) and also integrins expressed in leukocytes (αLβ2, αMβ2, and αXβ2), contain an insertion domain (I-domain) of 200 amino acid residues which is located between the second and the third domains [5, 10, 11]. This I-domain was also found in extracellular matrix proteins (collagen type VI), von Willebrand factor, and some complement factors [1]. Although in these proteins the I-domain is involved in protein–protein interactions, its functional role remains unclear in integrins. Taking into consideration the role of the I-domain in other proteins and lack of glycosylation site(s) in this region of integrins (and, consequently, acceptability for binding to other macromolecules) this domain is suggested to be involved in the interaction of these integrins with corresponding ligands [5, 11, 12]. Their binding involves a so-called MIDAS-site (metal ion-dependent adhesion site) located in the I-domain [13-15].

Many α-subunits (α3, α5, α6, α7, α8, αv, αIIb), lacking an I-domain undergo post-translational cleavage of the polypeptide chain followed by subsequent linkage of the two fragments by a disulfide bond (Fig. 1). The cleavage site is located at the 860th position (from the amino-terminus). Thus, the mature α-chain consists of larger amino-terminal and smaller carboxy-terminal fragments of molecular masses of 125 and 25 kD [1]. α4-Subunit represents the only exception; in this subunit the cleavage site is positioned closer to amino-terminus, and therefore the mature subunits consists of two roughly equal fragments (of 80 and 70 kD) linked by a disulfide bond.

The transmembrane domain of each α-subunit adopts α-helical configuration; it traverses the lipid bilayer (only once) and links the external and internal (cytoplasmic) domains. Among various α-subunits this region is characterized by the highest homology [1, 10, 16].

The cytoplasmic domains of α-subunits represent relatively short amino acid stretches of 20-50 residues that significantly differ in primary structure. The existence of several splicing variants increases the hetero-

geneity of cytoplasmic domains of α-subunits [2, 17]. Cytoplasmic domains play a primary role in ligand and signal properties of integrins, and variability of these sites is a basis for diverse functions of the whole integrin family and unique functions of certain receptors [6, 18].

1.2. Structure of β-subunits. The sizes of β-subunits vary from 95 to 117 kD. Primary structure of β-subunits is less variable than that of α-subunits. Although there are no genetic relations between α- and β-subunits, they share similarity in domain structure [1, 15, 19].

Each β-subunit contains a bivalent cation binding site that is located at the distance of 100 residues from the amino-terminus. As in the case of cation binding site of α-subunits, this site required for ligand–receptor interaction shares similarity with the EF-motif of Ca-binding proteins [1, 19].

Cysteine-rich (~20%) sequence located at the distance of 80 residues from the conservative site is a characteristic feature of all β-subunits [1, 19]. This region consists of four repeated fragments, each of which contains 45 residues, and 8 of them are cysteines. Cysteine residues are involved in the formation of disulfide bridges, which determine the tertiary structure of this region as a rigid stem [1, 8].

As in α-subunits the transmembrane domain of β-subunits adopts α-helical configuration and traverses the membrane only once.

The length of cytoplasmic domains of β-subunits varies in the range 15-65 amino acid residues. The cytoplasmic domain of β4-subunit is the only exception; it consists of about 1000 amino acids. In contrast to cytoplasmic domains of α-subunits, the cytoplasmic domains of β-subunits share high homology [20]. The existence of alternative splicing variants is the characteristic feature of cytoplasmic domains of β1-, β3-, and β4-subunits [2, 21-23]. The highest number of such variants was described for β1-subunit. All variants of cytoplasmic domains of β-subunits contain a conservative HDRR sequence flanking the transmembrane domain; this sequence forms a complex with the conservative sequence GFFKR of cytoplasmic domains of α-subunits and therefore participates in heterodimer assembly [2, 24, 25].

1.3. Tertiary structure of integrin dimers. Analysis of the human genome suggests the possibility of existence of 24 α- and 9 β-integrin subunits [26]. At the present time 18 α- and 8 β-subunits forming 24 heterodimers have been recognized [27]. Theoretically, they could form more than 100 heterodimers. However, in reality some limitations for dimer formation using certain α- and β-subunits exist. In most cases only one or a few α-subunits may bind certain β-subunit and these α-subunits do not form dimer(s) with other β-subunits (Table 1).

This feature of integrin dimer formation underlines integrin subdivision into separate β-subfamilies. The table shows that β1- and β2-subfamilies consist of 12 and four members, respectively. However, some α-subunits (e.g. α4, α6, αv)may form dimers with several β-subunits.

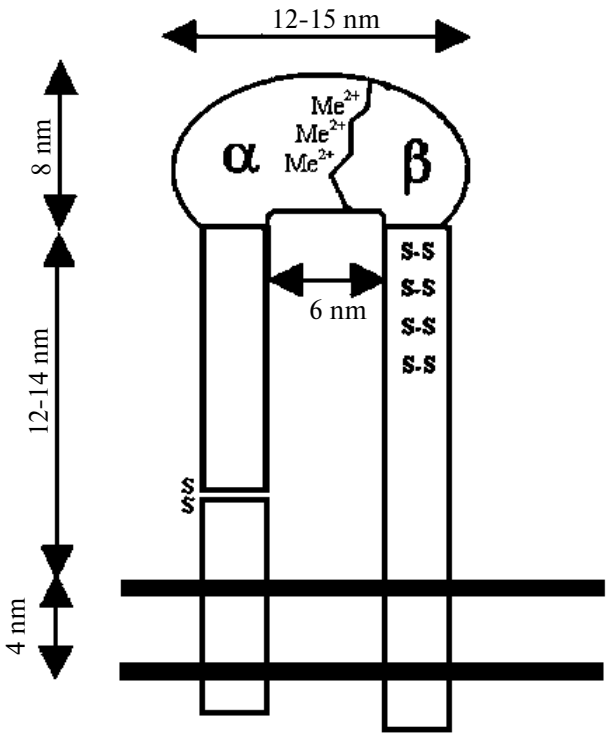

Fig. 2. Tertiary structure of an integrin dimer. See explanations in the text.

Figure 2 shows a schematic representation of the tertiary structure of an integrin dimer based on electron microscopy data. Both subunits of the dimer have a stem shape with extension (globule) at the amino-terminus. This extension is formed due to folding of the amino-terminus stabilized by intrapeptide disulfide bonds. Globular heads contain sites which provide (together with the above-mentioned cytoplasmic domain sequences) noncovalent binding of subunits into the dimer. These heads also contain repeated sequences that bind bivalent cations and form ligand binding site of the dimer [1, 2, 28]. Structural analysis shows that seven homologous repeats are folded into a seven-bladed propeller, generally representing one globular domain [2, 8, 29, 30]. Each "blade" contains four β-sheet structures and their connecting loops are located at opposite surfaces of this domain. It is suggested that the ligand binding site is located on one of these loops, and a bivalent cation binding site is located on the other loop [29, 31].

2. LIGAND SPECIFICITY OF INTEGRINS

Taking into consideration the positioning of integrin in the plasma membrane plane it is clear that the extracellular domain plays an important role in extracellular ligand binding.

Table 1. Ligand properties of integrins [1, 2]

Integrins		Ligands	Binding sites
β1	α1	Native collagen, laminin	
	α2	Native collagen, laminin	DGEA
	α3	Fibronectin, laminin, native collagen	
	α4	Fibronectin (splicing domain)	EILDV
	α5	Fibronectin (RGD-containing domain)	RGD
	α6	Laminin	
	α7	Laminin	
	α8	Fibronectin, vitronectin	RGD
	α9	Tenascin	
	α10	Collagen	
	α11	Collagen	
	αv	Vitronectin, fibronectin, osteopontin	
β2	αL	ICAM-1, ICAM-2, ICAM-3	
	αM	C3b, fibrinogen, ICAM-1, VCAM-1	
	αX	C3b	
	αD	ICAM-3, VCAM-1	
β3	αIIb	Fibrinogen, fibronectin, von Willebrand factor, vitronectin, thrombospondin	RGD, KQAGDV (fibrinogen)
	αv	Vitronectin, denatured collagen, von Willebrand factor, fibrinogen, thrombospondin, fibulin, osteopontin	RGD
β4	α6	Laminin, desmin	
β5	αv	Vitronectin	
β6	αv	Fibronectin	
β7	α4	Fibronectin (splicing domain),VCAM-1, MAdCAM-1	EILDV
	αE	E-cadherin	
β8	αv	Vitronectin	RGD

Note: Amino acids in binding sites are shown using single letter code; ICAM (intercellular adhesion molecule) and VCAM (vascular cell adhesion molecule) are surface receptors involved in intercellular connections; C3b is complement factor 3b; MAdCAM is the abbreviation of mucosal addressin cell adhesion molecule.

Matrix proteins were the first identified integrin ligands and fibronectin integrin α5β1 was the first identified matrix-specific receptor of the integrin family [32].

Comparative analysis of binding of intact fibronectin and its polypeptide fragments revealed a "key" ligand site that is ultimately required for binding with α5β1. This site was identified as an Arg-Gly-Asp tripeptide (RGD-peptide) [28, 33, 34]. Later it was demonstrated that many integrins bind corresponding ligands by their RGD-sites [1, 34].

Now, besides the RGD-site other oligopeptide sites responsible for binding with certain integrins have been identified in matrix proteins (Table 1). However, for many receptors specific binding sites have not been identified in corresponding ligands.

The data of Table 1 show that most integrins do not exhibit unique ligand specificity. Usually, each matrix protein may bind to several receptors and each integrin shows affinity to several of these proteins. It is hard to

interpret this multiple ligand specificity of the integrin family if integrins are considered only as "anchors" responsible for cell adhesion to the matrix substrate.

The development of ideas on integrins as components of cell signaling well explains this phenomenon. Certain evidence exists that the integrin receptors exhibiting common ligand specificity may transduce various intracellular signals controlling certain physiological reactions [35, 36].

Integral cell-surface proteins may act as ligands for integrins of some specialized cells. For example, leukocyte integrins αLβ2 and αMβ2 mediate leukocyte interaction with ICAM-1 and ICAM-2 receptors of an immunoglobulin family localized in the membrane of endothelial cells (Table 1).

It should be noted that data of Table 1 reflect only ligand potency of integrins that is not, however, realized in all cells. In a cell a certain receptor may be active or inactive with respect to a corresponding ligand and the receptor state depends on physiological conditions [37, 38].

3. SIGNAL FUNCTIONS OF INTEGRINS

The signal function of integrins can be defined as their ability to mediate the influence of extracellular matrix on intracellular processes modifying cell behavior. This influence is mutual and intracellular biochemical reactions, which employ integrins as a substrate, modify their conformation and, consequently, cell interaction with matrix. Thus, two pathways of signal transduction are generally recognized: outside-in and inside-out [3].

Historically, the physiological cell functions, regulated by integrin receptors, were initially revealed, and only later cytomorphological and biochemical processes involved in these regulatory mechanisms were clarified. In this review signal properties of integrins are considered in the same order.

3.1. Role of integrins in embryogenesis and cell differentiation and proliferation. In fibronectin knockout mice embryogenesis processes are impaired at the stage of mesoderm because of altered ability of mesodermal cells for adhesion, migration, and differentiation [39, 40]. Similar changes in embryonal development were also induced by mutations in the gene encoding the fibronectin-specific integrin α5β1 [41, 42]. In the latter case impairments were observed at later stages of embryogenesis. However, cells lacking α5β1 exhibited ability for adhesion and migration on fibronectin and they formed focal adhesions. These differences between fibronectin-deficient and receptor-deficient cells can be attributed to the interaction of fibronectin with integrins other than α5β1, which may compensate lack of the mutated receptor. In fact, defects of development similar (or even more dramatic) than those found in embryos lacking fibronectin gene were found in double mutants lacking α5 and αv genes ($\alpha5^{-/-}$ $\alpha v^{-/-}$). Other combinations of mutations in integrin genes ($\alpha5^{-/-}$ $\alpha3^{-/-}$, $\alpha3^{-/-}$ $\alpha4^{-/-}$, $\alpha4^{-/-}$ $\alpha5^{-/-}$) were not accompanied by such impairments [42]. This suggests similarity of signal pathways involving α5- and αv-receptors and their mutual functional replacement.

At present most integrins are more or less well characterized in terms of their role in embryonal and postembryonal development; this characterization is based on phenotypic deviations that appear after mutations in integrins genes [43]. The manifestations and degree of these impairments suggest both similarity and differences in functioning of individual receptors at various stages of development of an organism. For example, mice lacking a gene encoding α1 (and consequently not producing collagen specific integrin α1β1) maintained viability and fertility; however, these animals were characterized by dermal hypoplasia and reduction of proliferating activity of cells [44]. However, mouse embryos deficient in the gene encoding α2 and lacking the other collagen-binding integrin α2β1 die at the stage of implantation [43].

Some integrins have marked tissue specificity and exhibit biological activity in certain tissues at certain stages of embryogenesis and postnatal development. Integrin α7β1 is a receptor for three types of laminin; it is expressed mainly in skeletal muscle and myocardial cells. Mice lacking the gene encoding the α7-subunit were viable and fertile. However, soon after birth symptoms of a progressive muscular dystrophy caused by impairments in function of the myotendinous junctions were recognized [45-47]. In the case of deficit of α3β1 and α6β1 integrins, which are widely present in various tissues, severer defects were observed during embryonal development. Mice lacking the α3 integrin gene died soon after birth due to abnormalities in kidneys, lungs, and central nervous system [48-50]. α6-Intergin knockout mice also die soon after birth due to symptoms of severe impairments in skin, brain, and retina [51-53].

The involvement of integrin in differentiation was studied using keratinocyte culture under conditions of so-called terminal differentiation, which was induced by cell culturing in suspension when surface receptors are inactive and not ligand bound. Under these conditions, down-regulation of β1 RNA was observed and β1-containing receptors disappeared from the cell surface [54]. These changes indirectly suggest that β1-integrins inhibit keratinocyte differentiation in suspension. This effect was rather specific because it was not observed in suspension culture of other cell types and it did not involve other surface receptors of keratinocytes. The role of β1 integrins was demonstrated more clearly by comparing differentiation markers (keratins) in two lines of mouse embryonal cells, one of which was from wild type embryos, whereas the other one was from embryos with mutated β1 integrin gene [55]. Starting from a certain stage the mutated cells

(but not wild cells) stopped expression of the differentiation markers.

Transfection of cDNA of various α-integrin subunits into cultured myoblasts yielded strong evidence of the specific role of some integrins in mechanisms of cell differentiation [56]. In the initial (intact) cells signs of differentiation appeared at the confluent stage. Cells overexpressing α5-integrin subunit actively divided without any signs of differentiation even at the confluent stage. Cells overexpressing α6-integrin subunit do not proliferate; they are characterized by a high degree of differentiation. Study of cells expressing chimeric subunits (containing extracellular domain of α5 in combination with cytoplasmic domain of α6 or extracellular domain of α6 fused with cytoplasmic domain of α5) revealed specific role of the cytoplasmic domain of α6-subunit in induction of differentiation.

Involvement of integrins in cell proliferation has been demonstrated using various experimental approaches [57, 58]. Most normal cells do not divide in the unanchored state, and their division begins only after cell anchorage on a solid substrate. This phenomenon has been known for a long time as substrate-dependent growth [59]. The transition of a cell from a resting to proliferating state is characterized by a shift of acid–base status to pH increase. Certain evidence exists that ligand-induced clustering of α5β1 integrin causes activation of Na^+/H^+ antiport [60].

Integrins involved into mechanisms of proliferation act synergistically with growth factor receptors exhibiting tyrosine kinase activity (RTK, receptor tyrosine kinase). Activation of integrins induced by their clustering on immobilized antibodies or a natural substrate resulted in accumulation and activation of RTK in integrin clusters [61]. Both types of receptors are involved in Ras and MAPK (Mitogen Activated Protein Kinase) signaling pathways triggering kinase cascades. Products of these cascades enter the nucleus and activate transcription factors controlling DNA replication [62, 63]. There is clear interrelation between a mitogenic signal and a type of integrin realizing this signal. For example, in tenascin-anchored fibroblasts induction of MAPK activity and proliferation of fibroblasts was observed only when cell adhesion was mediated through integrin αvβ3 and α9β1 receptors; when adhesion of the same cells on the same substrate was mediated through integrin αvβ6 this effect was absent [64].

The involvement of integrins in mechanisms of cell proliferation may also be illustrated by integrin-dependent changes of functional activity of genes controlling the mitotic cycle in various cell types [57, 65]. Expression of cyclins A and D1 and their mRNA depends on matrix mediated receptor activation [66-69].

3.2. Organization of focal adhesions. In cells unattached to a substrate integrins are diffusely distributed in the plasma membrane and they do not exhibit signal activity. Interaction with matrix proteins causes morphological changes of the plasma membrane and of cell fibrillar cytoplasm components, cytoskeletal structures. These rearrangements result in formation of focal adhesions. The focal adhesions contain matrix proteins at the extracellular sites and microfilaments complexed with actin-binding proteins at the cytoplasmic sites (Fig. 3).

Focal adhesions are recognized only in cell cultures. *In vivo* their morphological and functional analogs are smooth muscle sarcolemma dense disks [70]. Integrin binding to ligand is accompanied by formation of a bridge between matrix and actin cytoskeleton and integrins are concentrated into clusters that aggregate in the focal adhesion [2, 71, 72]. Under these conditions integrins exhibit signal activity.

Focal adhesions play important roles. It is an anchor fixing actin microfilament bundles involved in morphogenetic cell reactions (spreading onto substrate, formation of filopodia and lamellopodia, endocytosis, etc.) and cell locomotion [59, 73-75]. The other role consists of concentration of messengers transducing signals from integrins and other cell receptors to the genome [2, 4, 76].

Adhesion formation is an integrin-dependent process. For example, fibroblast attachment on fibronectin induces focal adhesion formation; however, this effect is not observed during adhesion on concanavalin with which integrins do not interact [77]. It should be noted that focal adhesions can be formed during cell attachment not only on matrix proteins but also on other substrates that can bind integrins, for example, immobilized anti-integrin antibodies. Such an approach is often used for specific activation of an individual receptor [78, 79].

The composition of focal adhesions and activity of their macromolecular constituents depend on physiological conditions and they can vary even within one cell [74, 80, 81]. Figure 3 shows one possible variant of composition and interaction of macromolecules in the focal adhesion. Many of the macromolecules identified in the focal adhesions are not shown [2, 74].

There is certain sequence of transport of cytoskeletal proteins and signal molecules to the plasma membrane region. The clustering of integrins induces recruitment of structural cytoskeletal proteins, vinculin and talin, which interact with actin and promote its polymerization into microfilaments. At a later stage, α-actinin is included into focal adhesions. α-Actinin stabilizes mature filaments by forming cross-links with adjacent bundles and promotes their fixation in the membrane due to interaction with cytoplasmic domains of integrin β-subunits. Other cytoskeletal proteins that link integrins with microfilaments play a similar role. For example, talin forms a bridge between the cytoplasmic domain of the integrin β-subunit and actin through vinculin, or vinculin–tensin, and also through paxillin–vinculin [2, 74]. Filamin links integrins with microfilaments [82].

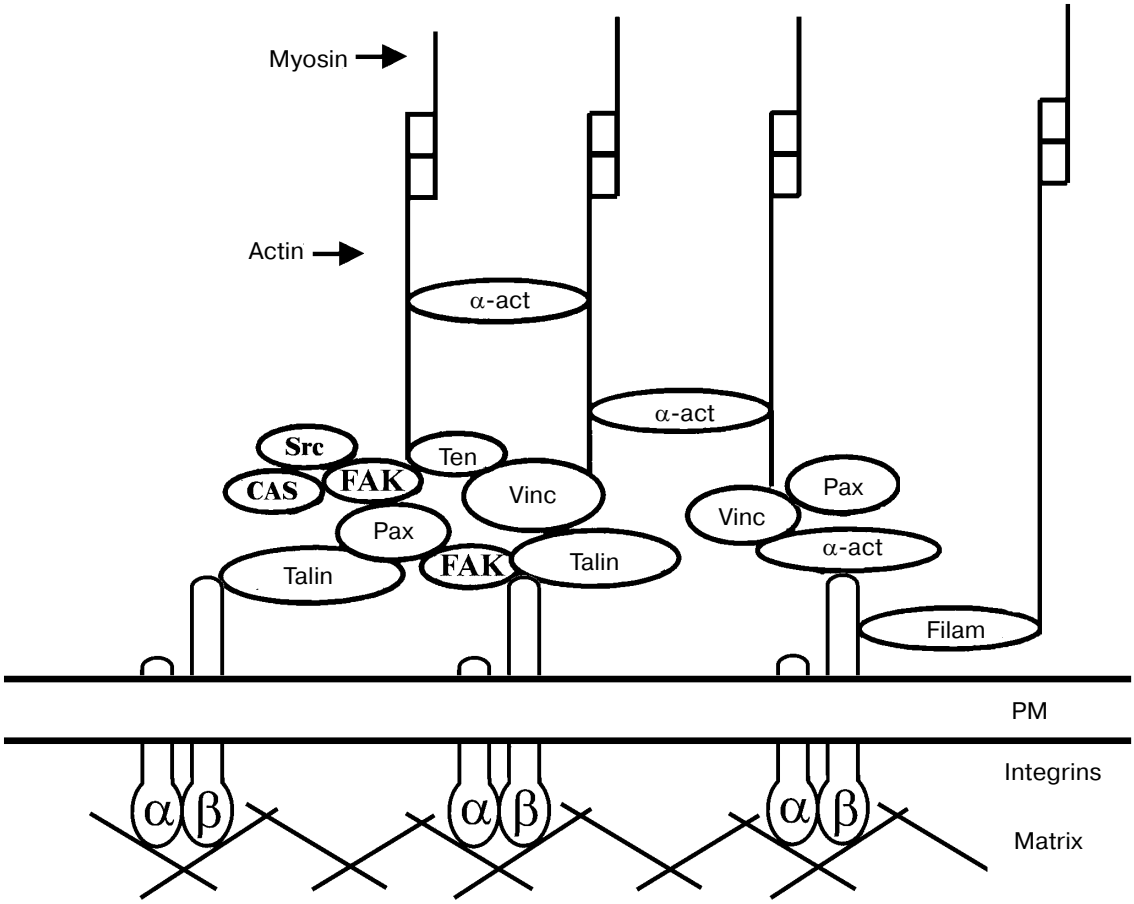

Fig. 3. Structure of the focal adhesion. Abbreviations: α-act) α-actinin; Ten) tensin; Src) protooncoprotein c-Src; FAK) Focal Adhesion Kinase; CAS) protein $p130^{Cas}$; Vinc) vinculin; Pax) paxillin; Filam) filamin. Other explanations are in the text.

Biogenesis of focal adhesions includes an earlier stage that is characterized by formation of so-called focal complexes. These complexes consist of small integrin clusters that form relatively weak bonds with substrate. At a later stage (of mature focal adhesions) actomyosin fibers known as stress filaments are formed. Isometric tension developed in these stress filaments attracts additional clusters of integrins into the focal complexes. This process is accompanied by conformational changes in integrin dimers, increase in their affinity for matrix substrate, and formation of tight mature adhesions [74, 83]. In these reactions small cytoplasmic G-proteins known as GTPases of the Rho family play the key role. Functions of these proteins are considered in Section 3.3.

3.2.1. Role of the cytoplasmic domain of integrin β-subunits. As mentioned above, sizes of cytoplasmic domains vary within 20-60 amino acid residues. This is about 3% of the dimer size. Nevertheless, all integrin-mediated signals controlling various cell functions pass through these sites. Cytoplasmic domains are fixed within the cell surface region. This suggests the existence of many cytoplasmic messengers that could associate with them.

In fact, several tens of cytoplasmic proteins interacting with integrin cytoplasmic domains are now known. These include actin cytoskeleton proteins, adaptor proteins, phosphokinases, chaperons, transcriptional cofactors, and other proteins with unknown functions [2, 73].

Table 2 shows that in most cases cytoplasmic proteins bind to the cytoplasmic domain of the β-subunit. This is consistent with data on a central role of cytoplasmic domains of β-subunits in organization of cytoskeleton and focal adhesions and also in signaling. For example, mutant receptors of β1, β2, and β3 families lacking the cytoplasmic domain of the β-subunit were unable to form clusters; these receptors exhibited lower activity in ligand binding and activation of downstream signal messengers [73, 84, 85].

Table 2. Integrin cytoplasmic domain binding proteins [2, 73, 86]

Protein	Integrins	Function
Cytoskeleton binding proteins		
Talin	β, αIIb	Focal adhesion protein
Actin	α1, α2	Microfilament formation
Filamin	β1, β2, β7	Cross-links between filaments
α-Actinin	β1, β2	Cross-links between filaments
Myosin	β3	Actin filament contraction
Plectin	β4	Intermediate filaments binding
Adaptor and signal proteins		
Shc	β3, β4	Contains SH2-domain; growth factor receptor binding
Grb2	β3	Contains SH2- and SH3-domains; binding to phosphotyrosine and proline-enriched sites
RACK1	αv, α4, β1, β2, β5	Binds protein kinases C and Src
Paxillin	α4, α9, β1, β3	Focal adhesion protein; contains SH2- и SH3-domains; binding to phosphotyrosine and proline-enriched sites
Cytohesins (1, 2)	β2	Stimulate cell adhesion
Protein kinases		
Focal adhesion kinase, FAK	β1, β2, β3	Activation in response to binding of integrins and ligands of growth factor receptors; binds Src, Grb2, paxillin, $p130^{Cas}$ and PI-3-kinase
Integrin-linked kinase, ILK	β1, β3	Serine/threonine kinase, localized in focal adhesions; phosphorylates β1-integrins and AKT- and GSK-3β protein kinases
Chaperones		
Calnexin	α6, β1	
Calreticulin	many α	Ca^{2+} import
Transcriptional cofactors		
JAB1	β2	Binds Jun
BIN1	α3	Tumor suppressor, binds Myc

In other studies the role of the cytoplasmic domain of β-subunit was demonstrated by transfection into mouse fibroblast of intact and modified chicken β-subunit followed by subsequent determination of localization of cytoskeletal proteins [87]. Deletion of 15 C-terminal amino acids in the cytoplasmic domain of β1-subunit suppressed co-localization of actin, talin, and α-actinin with integrin clusters.

Fibronectin attached fibroblasts expressing chimeric proteins that consisted of extracellular domain of interleukin (this protein does not form bonds with matrix) and cytoplasmic domain of β1-subunit or α5-subunit "concentrated" in the focal adhesions only proteins containing β1-cytoplasmic domain. This suggests that the cytoplasmic domain of β-subunits carries ligand-independent information for receptor driving into the focal adhesions [88].

Among cytoskeletal proteins, talin and its role in integrin functioning have been studied in more detail. Overexpression of a fragment of the talin molecule carrying a site for binding of cytoplasmic domain of β-subunit increased ligand activity of αIIbβ3 integrin in CHO cells [89]. Reduced expression of talin inhibited export of integrins from Golgi complex, their expression in membrane, and focal adhesion formation [90, 91]. Mutations in talin-binding site of β-subunit cytoplasmic domain cause impairments in translocation of talin and actin into focal adhesion without any changes in integrin clustering [73].

The other well characterized cytoskeletal proteins forming complexes with cytoplasmic domains of β-subunits are α-actinin and filamin [73]. Although they form less tight complexes with β cytodomains than talin, studies in cell cultures and cell-free systems revealed, that recruitment of these proteins into focal adhesions ultimately requires their association with these domains [92]. Mutations in cytoplasmic domain loci of β1- and β2-subunits responsible for α-actinin binding resulted in impairments of formation of focal adhesions and stress filaments. Mutation analysis of sites responsible for talin and α-actinin binding revealed that these proteins have different functions in the cell, but all these functions depend on β cytoplasmic domains [73, 93].

Like α-actinin, filamin is localized not only in focal adhesions but also along actin filaments. Its functions are related to mechanical stress of cells during changes of their shapes, formation of filopodia, and motility [82]. Mutations in the filament-binding site located in the C-terminal region of β cytodomains cause impairments of integrin assembly in focal adhesions [94].

3.2.2. Role of cytoplasmic domains of integrin α-subunits. Although results of most studies show the preferential role of cytoplasmic domain of β-subunits in focal adhesion organization, recent data suggest α-subunit involvement in this process. For example, deletion of cytoplasmic domain of α1-subunit of the collagen-specific integrin α1β1 caused loss of focal adhesion forming ability during cell culturing on collagen, whereas their plating on fibronectin resulted in focal adhesion formation and cell spreading. It was shown that α1-subunit containing intact cytoplasmic domain binds talin, α-actinin, and paxillin, whereas α1-subunit lacking this domain cannot bind these proteins [6]. Another study identified binding sites in cytoplasmic domain of α4-subunit and paxillin required for complex formation between these proteins. The functional importance of these complexes was demonstrated by blockade of interaction between α4-subunit and paxillin, which caused inhibition of cell migration on the substrate [95].

In contrast to cytoplasmic domains of β-subunits, the cytoplasmic domains of various α-subunits are characterized by greater diversity of their primary structure (with the exception of the GFFKR sequence, see Fig. 1) [1, 2]. This suggests the existence of specific functions of cytoplasmic domains of certain α-subunits. For example, α4-integrins differ from other receptors by their stimulation of cell migration and inhibition of cell spreading and focal adhesion formation [96, 97]. These effects are determined by cytoplasmic domain of α4-subunit, because expression of chimeric receptors containing cytoplasmic domain of α4-subunit in "foreign" α-subunit resulted in stimulation of cell migration and inhibition of focal adhesion formation [97, 98].

The role of the cytoplasmic domain of α-subunit was demonstrated during study of adhesion of fibroblasts on fibronectin and collagen; this study employed fibroblasts expressing intact integrin α5β1 in combination with intact α1β1 receptor or α1β1 receptor lacking the cytoplasmic domain of the α-subunit [99]. It was shown that both intact and modified α1β1 were found in focal adhesions on collagen (which is specific ligand for α1β1). However, in focal adhesions, formed on fibronectin, α5β1 (specific for this ligand) and modified (but not intact) α1β1 were found. Thus, the intact cytoplasmic domain of the α-subunit involves ligand-specific integrin recruitment into the focal adhesions, whereas in the absence of cytoplasmic domain ligand-independent receptor recruitment into focal adhesions occurs.

According to the modern viewpoint, when receptor is not bound to a ligand the cytoplasmic domain of the α-subunit somehow blocks interaction of cytoplasmic domain of the β-subunit with cytoskeleton. Ligand binding causes some conformational changes of these sites, which cancels this blockade. Thus, the cytoplasmic domain of α-subunit is responsible for specific receptor activation (which depends on a protein matrix ligand), focal adhesion formation, and signaling. Mutation analysis revealed that these conformational rearrangements require conservative sequences GFFKR and HDRR in membrane flanking sites of α- and β-subunits, respectively (Fig. 2) [73, 100].

3.3. Mechanisms of integrin-dependent intracellular signaling. Understanding of the role of integrins as com-

ponents of the intracellular signal system was formed after discovery of a common signaling mechanism (mediating effects of many receptors), which involves cascades of phosphorylation reactions catalyzed by intracellular phosphokinases [62, 69, 74].

It was found that integrin aggregation into clusters not only induced focal adhesion assembly involving cytoskeletal proteins but also caused concentrating of some phosphokinases in these structures. These phosphokinases trigger sequential phosphorylation of proteins exhibiting specific functions in the cell. Several protein substrates undergoing integrin activated phosphorylation include proteins involved in focal adhesion formation (e.g., tensin and paxillin) [62, 101]. Integrins also induce phosphorylation of protein kinases located in focal adhesions (FAK, Src, Abl, etc.) required for their activation [69, 76, 102].

3.3.1. Role of focal adhesion kinase (FAK). Since integrins did not exhibit any catalytic activity but did stimulate focal adhesion protein phosphorylation, the existence of a special integrin-activated phosphokinase catalyzing these reactions was proposed. In fact this enzyme is known as focal adhesion kinase (FAK). FAK is a protein of 125 kD catalyzing phosphorylation of tyrosine residues in protein substrates.

FAK plays a key role in transduction of integrin-mediated signals. For example, mouse embryos with knocked out gene encoding fibronectin or FAK die at the gastrula stage due to similar developmental defects [64].

Details of mechanism responsible for integrin-dependent activation of FAK remain unknown. However, co-localization of integrins and FAK in focal adhesions, integrin-induced FAK phosphorylation, and the existence of noncatalytic FAK domain binding site in cytoplasmic domain of β1-subunit seem to support the involvement of integrin in FAK activation [62, 103]. Overexpression of chimeric proteins containing cytoplasmic domains of β1-, β3-, or β5 fused with non-signaling extracellular domain stimulates FAK phosphorylation [104].

Integrin clustering induces concentrating of FAK in focal adhesions; this causes enzyme autophosphorylation at tyrosine-397 [62, 105]. The phosphorylated Tyr397 binds to SH2-domain (Src homology domain) of the protooncoprotein c-Src, which also (like FAK) is a non-receptor tyrosine phosphokinase. Binding to FAK activates Src-kinase, which catalyzes phosphorylation of other tyrosine residues in the FAK molecule. This results in complete activation of FAK. At this stage the adaptor protein $p130^{Cas}$ binds to FAK–Src complex [106].

FAK activation is accompanied by numerous effects resulting from diversity of signaling pathways employing this enzyme [69, 105].

One such pathway includes transduction of signals controlling cell proliferation (Fig. 4) [69]. This is initiated by binding of the adaptor Grb2 protein, which belongs to a group of growth factor receptor binding proteins, to phosphorylated Tyr925 of FAK. Grb2 activates SOS protein catalyzing guanine nucleotide exchange in Ras protein. The substitution of GDP for GTP results in conversion of inactive Ras-GDP into the active RAS-GTP form. The activated Ras protein triggers the MAP-kinase cascade, which includes sequential activation of serine/threonine type kinases Raf, MEKK, and Erk (MAPK). The latter is translocated into the nucleus where it phosphorylates and activates transcription factors that activate genes responsible for proliferation [63, 105, 107]. It should be emphasized that the pathways of integrin-mediated (substrate-dependent) proliferation and growth-factor regulated proliferation converge at the stage of Grb2-Ras. However, these pathways are not interchangeable. For example, high level of inhibitors of cell proliferation, proteins $p21^{WAF1/CIP1}$ and $p27^{KIP1}$, and corresponding inhibition of proliferation were observed in both substrate anchored cells lacking growth factors and in suspended cells in the presence of growth factors. Each of these signaling pathways contributes to the mitogenic effect that is especially stable on combined functioning of these pathways [69, 104].

Two other pathways shown in Fig. 4 transduce signals controlling change of the shape of cells and their interrelationships with substrate (adhesion, spreading, and motility) [57, 62, 108, 109]. Both cascades are initiated by interaction of activated $p130^{Cas}$ with adaptor protein Crk.

The Cas–Crk complex activates protein C3G factor responsible for transition of inactive GDP-bound form of Rap1 GTPase (Ras protein analog) into the active GTP-bound form. The function of Rap1 consists of polarization of actin cytoskeleton and stimulation of cell spreading onto the substrate [62, 110].

The second signaling pathway controls cell locomotion. It is related to functioning of Cdc42 and Rac proteins. These G-proteins are members of the Rho family. Rac functions have been investigated in detail. Activation of this and other GTPases is mediated by some unidentified factor responsible for guanine nucleotide exchange (Fig. 4). Perhaps this function can be attributed to SOS factor and/or one of the members of the c-Vav protooncoprotein family [83, 111, 112]. Subsequent signal transduction from Rac to a system responsible for cell migration represents an interesting example of concerted regulation of signal molecules [108, 109, 113-115].

Cell migration along a substrate consists of alternative incompatible morphogenetic reactions each of which is activated by a separate GTP-binding protein (G-protein) of the Rho family.

Rac GTPase determines the mobile stage (protrusion), which is characterized by cell spreading, actin polymerization at the leading edge of migrating cells, and formation of ruffles and lamellopodia. This function involves several messengers for signaling from Rac to Arp2/3. This complex initiates polymerization of actin on the preformed filaments followed by formation of a

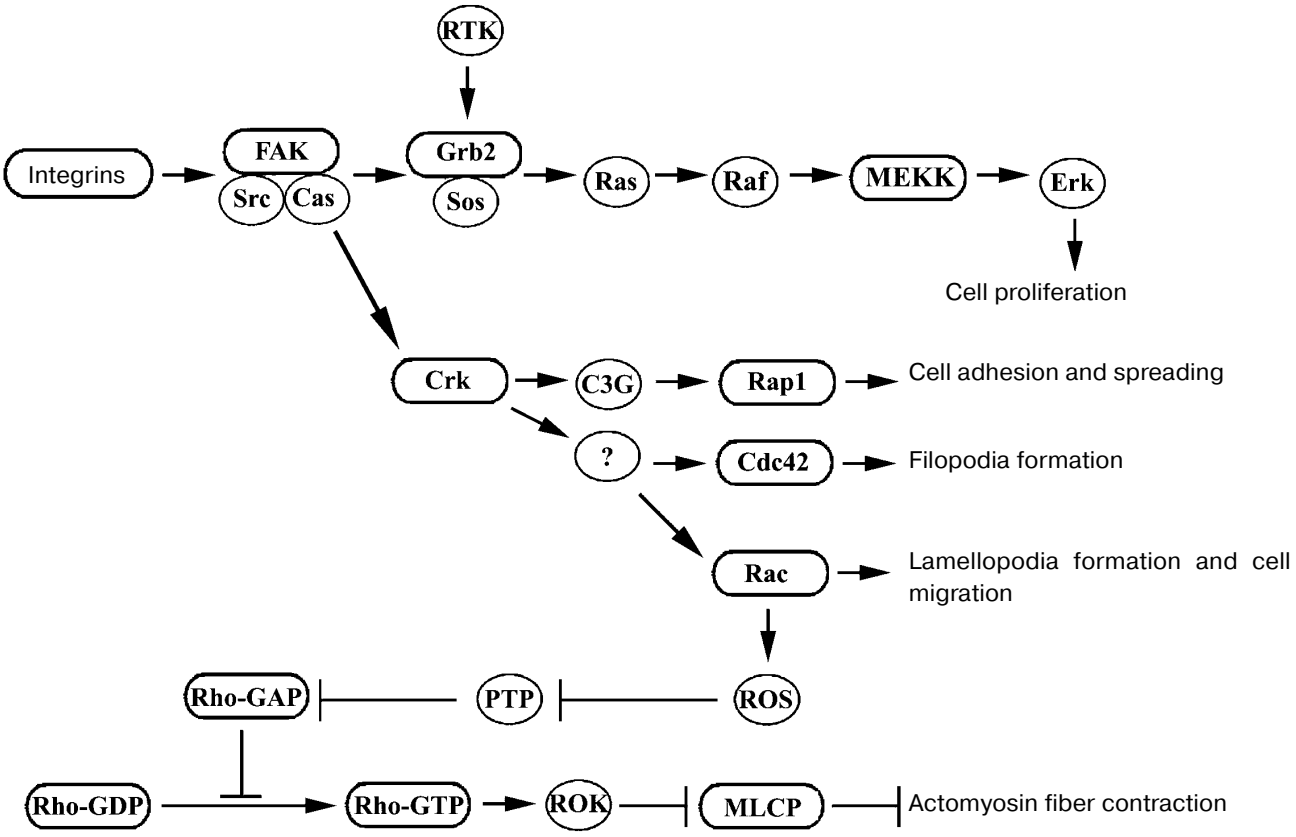

Fig. 4. Scheme for integrin-mediated signaling. See explanations in text.

branched actin network constituting the basis for lamellopodia [111]. At this stage, precursors of focal adhesions, focal complexes, containing a relatively small amount of integrin clusters are formed in the region of lamellopodial membranes. At the stage of protrusion, depolymerization of actomyosin fibers (stress filaments) and decomposition of "old" focal adhesions occurs [75, 111]. Rac activity, rate of lamellopodia formation, and rate of cell migration depend on the level and composition of integrins at the leading edge and also on the composition of surrounding matrix [83, 111].

The next stage (contraction) controlled by Rho GTPase consists of formation of stress filaments, rounding and contraction of cell body, and detachment of its "tail" from the substrate [111].

Since these phases are antagonistic some mechanism is required for the sequential switch of one phase to another. In fact, such a mechanism has recently been described [109, 115, 116]. It has been demonstrated that in parallel and irrespectively to signals stimulating lamellopodia formation Rac reversibly inhibits Rho protein signaling; this is mediated by reactive oxygen species. Details of this signaling pathway are shown in Fig. 4.

The active form of Rac (Rac-GTP) stimulates plasma membrane NADPH oxidase. Reactive oxygen species (ROS) formed in the reaction catalyzed by this oxidase (particularly, superoxide radical) inhibit phosphotyrosine phosphatase (PTP) by oxidizing active site cysteine residues. This promotes phosphorylation and activation of its substrate, Rho-GAP (GTPase activating protein), which blocks formation of the Rho active form, Rho-GTP. Lack of this form is accompanied by accumulation of inactive form of ROK (Rho kinase), a serine/threonine type protein kinase. Decrease of ROK decreases phosphorylation of its substrate, myosin-binding subunit (MBS) of myosin light chain phosphatase (MLCP), and decrease of MBS phosphorylation results in increase in MLCP activity, decrease in myosin light chain phosphorylation, and blockade of contractility of actomyosin fibers [75, 111].

Termination of Rac signaling automatically causes Rho activation, actin polymerization, and formation of actomyosin fibers. Morphogenetic manifestations of these reactions depend on types of cells and their physiological conditions. In actively migrating cells tension developed in actomyosin fibers results in pulling of the cell body towards the leading edge, and during cell migration focal adhesions disorganize. Upon canceling of movement, the tension of actomyosin fibers causes integrin clustering at the focal adhesions and increase of

adhesive bonds between receptors and matrix [111, 117]. It is important that locomotive activity of cells inversely depends on tightness of integrin-mediated bonds between matrix and cells, and tightness of these bonds directly correlates with activity of Rho protein [118]. Rho protein increases tightness of these bonds not only by stabilizing the intracellular part of the focal adhesions, but also by influencing integrin conformation and increase in their affinity to matrix proteins, i.e., due to signals directed outside the cell [83].

Thus, cell motility is a cyclic process in which well coordinated alternation of mutually regulated integrin mediated "outside-in" and "inside-out" signals play the key role.

3.3.2. Role of other phosphokinases. *Protein kinase C (PKC).* The family of serine/threonine protein kinases C (PKC) is involved in integrin-mediated signaling [7]. Fibronectin binding to integrins results in recruitment of PKC to the plasma membrane region, where PKC participates in focal adhesion formation, FAK phosphorylation, and cell spreading [119, 120]. PKC binds integrin β1-subunits; this involves tetraspanins that act as bridges between the cytoplasmic domain of the β1-subunit and the enzyme [121]. In another study, PKC stimulated expression of β1 integrin on cell surface, their endocytosis and intracellular transport, and increased cell migration [122]. Using carcinoma cells as a model, it was demonstrated that PKC activation was important for integrin α6β4 mobilization from hemidesmosomes into lamellopodia, where this receptor was ultimately required for locomotive activity of epithelial cells [123].

Protein kinase PAK (p21-activated kinase). The family of serine/threonine protein kinases, PAK, links substrate-dependent and RTK-dependent activation of ERK [69]. PAK translocation to the plasma membrane (where it is activated by Rac G-protein) involves association of this enzyme with SH2/SH3-containing adaptor Nck protein [124, 125]. The effect of PAK on integrin- and RTK-dependent signal transduction is realized via direct phosphorylation of Raf and MEK kinases (catalyzed by this enzyme). Raf kinase and MEK are common components for these signaling pathways. Being the effector of Rac protein, PAK is involved in antagonism between Rac and Rho, because it catalyzes phosphorylation of myosin light chain kinase, which results in inhibition of actomyosin filament contractility.

Phosphatidylinositol-3-kinases (PI3K) also represent a family of phosphokinases that are activated during the interaction of integrins with matrix [7, 127]. The combination of lipid kinase and protein kinase activities is a characteristic feature of these enzymes. Interaction of SH2-domain of PI3K catalytic subunit with FAK and Src phosphotyrosines results in translocation of PI3K to the plasma membrane, and this precedes their activation. Recruitment and activation of PI3K also involve RTK (receptor tyrosine kinases) and Ras protein [128]. PI3K catalyzes phosphorylation of inositol-containing phospholipids by the D3 hydroxyl group of the inositol ring; this results in formation of phosphoinositides PI(3)P, PI(3,4)P2, and PI(3,4,5)P3. Phosphorylation products share affinity to PH-domain (pleckstrin homology domain) of some intracellular proteins and recruit them to the plasma membrane where these proteins become activated. Many of these proteins are components of important biological mechanisms, and this explains the diversity of processes controlled by PI3K. Signaling cascades initiated by PI3K are involved in regulation of cell proliferation, locomotion, vesicular transport, apoptosis, and oncogenesis [7, 128-130]. A feedback mechanism exists between PI3K and integrins, and in some cases receptor activation requires catalytic activity of this enzyme [131].

Integrin mediated cell interaction with matrix also contributes to activation of phosphatidylinositol-5-kinase, catalyzing phosphorylation of phosphoinositide-4-phosphate. The reaction product, phosphoinositide-4,5-bisphosphate, interacts with actin-binding proteins, profilin and gelsolin; this causes release of actin monomers and promotes their polymerization into microfilaments [111].

ILK (integrin-linked kinase). This serine/threonine type protein kinase is the only kinase with perfectly documented direct interaction with integrins [132-134]. ILK catalyzes phosphorylation of Ser790 in the cytoplasmic domain of the integrin β1-subunit; this is required for receptor localization in focal adhesion [132]. This kinase also initiates formation of a branched network of protein–protein interactions mediating integrin links with many structural and signaling proteins, which extend their signaling functions [134]. This regulatory property of ILK is associated with the existence of several domains exhibiting affinity to various types of biological molecules. Four repeated ankyrin sequences located at the N-terminus are responsible for ILK binding with PINCH, forming associates with growth factor receptors and PI3K. The PH-domain located in the central part of the ILK molecule links this enzyme with PI(3,4,5)P3. The catalytic (kinase domain) located at the C-terminal region can bind to the cytoplasmic domain of integrin β1- and β3-subunits, and this determines ILK localization at focal adhesions [133-135]. Other proteins (affixin, paxillin, CH-ILKBP) forming associates with actin also bind to this domain [126].

Thus, the association of ILK with integrins initiates formation of a multicomponent complex, and this represents a structure forming function of ILK, which is important for stabilization of a focal adhesion and its interaction with cytoskeleton.

Also, ILK links with signal molecules of this complex are a basis for the signaling functions of this enzyme. ILK activation is initiated by integrins, growth factors, which

act via RTK. Integrin- and RTK-dependent stimulation of ILK is controlled by PI3K via PI(3,4,5)P3 [136].

ILK phosphorylates Akt/PKB and GSK-3 (glycogen synthase kinase-3), and this causes opposing effects: activation of Akt/PKB and inhibition of GSK-3 [133, 136]. The physiological importance of these signals is determined by the key role of these kinases in cell survival and regulation of cell cycle [132, 134]. For example, expression of dominant-negative form of ILK caused arrest of cell cycle at G_1 and the development of apoptosis [137]. This corresponded to a decrease in Akt/PKB activity, playing one of the key roles in protection of cells against apoptotic death [63, 127]. The most pronounced effect was observed in cells lacking either links with matrix or growth factors [137].

The effect of ILK on cell cycle is determined by its inhibitory action on GSK-3. The mechanism underlying this effect involves binding of E-cadherin (receptor of cell surface) with β-catenin. After cleavage of this bond β-catenin forms a complex with Lef1 transcription factor. This complex is further translocated into the nucleus where it activates transcription of genes encoding cyclin D1 and oncoprotein Myc [63]. Phosphorylation of β-catenin catalyzed by GSK-3 stimulates its degradation and consequently prevents formation of active transcription complex Lef1/β-catenin [63, 138]. Thus, ILK-induced inhibition of GSK-3 promotes stabilization of β-catenin and, finally, stimulates the cell cycle and proliferation.

This effect of ILK is potentiated by inhibitory action of Lef1/β-catenin on expression of E-cadherin, which "deprives" β-catenin its partner on the cell surface and promotes its translocation into the cytoplasm and then into the nucleus [133, 138]. Decrease in E-cadherin in the cell membrane also destabilizes intercellular interactions.

4. CONCLUSION AND PERSPECTIVES

Integrins are involved in various physiological processes. The role of these receptors is determined by their topography, favoring direct simultaneous interaction with extracellular matrix and cytoskeleton, and also by the biochemical properties of integrins.

The discovery of the main principle of integrin functioning, ability for lateral migration in the membrane plane and multifold increase in adhesive and signaling activities during cluster formation and decrease in these activities after cluster dissociation was an important achievement of modern cell biology. Such a "regime" provides cycling of cell reaction and alternation of "divergent" processes, adhesion, division, motility.

An intriguing property of integrins is their diversity in parallel with the fact that each receptor is characterized by ligand cross-reactivity. One plausible explanation of this "excess" may consist in the fact that natural integrin substrates, matrix proteins, often share similarity in primary and other levels of their organization (various types of collagen represent a typical example). However, under certain conditions these substrates generate various signals. It is possible that these signals are detected by structurally related integrins, which, however, may recognize different sites. Data considered in Section 3.1 seem to support such possibility. Thus, certain evidence exists that this "excess" just demonstrates limits in modern knowledge about integrin binding sites in ligands and ligand binding sites in integrins. Subsequent studies should solve this problem.

The other surprising fact consists of the number of intracellular proteins that can bind such relatively short peptide fragments as cytoplasmic domains of integrin subunits. This property may reflect diversity of signal cascades that are mediated by integrins. However, molecular mechanisms of this phenomenon, and, particularly, structure of recognition sites in cytoplasmic domains and in their protein ligand represent an interesting subject for future studies.

In this review we have considered the main signaling pathways and basic behavioral cell reactions that involve integrins. The role of these receptors in cell proliferation, differentiation, and motility, the diversity of extra- and intracellular proteins interacting with integrins, and diversity of signaling cascades mediating integrin effects determine integrin involvement in the development of various pathological states. These include oncogenesis, malignant progression of tumors, inflammation, impaired reaction of immune response, and apoptotic cell death. In each field many interesting results important for understanding of these processes have been obtained, and progress in integrin study will make substantial contributions to subsequent success in final elucidation of these mechanisms.

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