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Review

Focal Adhesion Kinases

Interest in Immunoendocrinology, Developmental Biology, and Cancer

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The research field on focal adhesion–related kinases started a decade ago, but the term *focal adhesion* was introduced for the first time nearly 20 yr before. Since its identification, many studies have enlightened the role of the first intermediate of focal adhesion–related signals in a large number of biologic and physiologic processes. In this review, we try to integrate the most recent data about the known focal adhesion–related kinases, and we focus on three topics in which they deserve great interest: neuroendocrine-immune interactions, developmental biology, and proliferative diseases.

Key Words: Focal adhesion; $p125^{FAK}$; T lymphocytes; cancer.

Introduction

As a biologic event, adhesion means either cell-to-cell adhesion or adhesion to extracellular matrix (ECM). Cell-to-cell junctions can be adhesive (desmosome), closed (tight junctions and invertebrate septa), or communicative (gap junctions and synapses); cell adhesion to ECM is described in morphologic terms such as inclusive, adhesive plaques, or focal adhesion. The term *focal adhesion* was introduced by Abercrombie et al. *(1) i*n 1971 to describe an in vitro event—the binding of fibroblasts to ECM—and defined a transitory adhesion of a mobile cell to a matrix substrate. Thus, this definition primarily relied on a morphologic observation, but today it is no longer appropriate because most of the molecules implicated in focal adhesion also mediate signaling pathways. Moreover, this definition does not take into account different morphologic observations such as adhesion plaques or contacts between T lymphocytes and thymic epithelial cells (TECs) that can be viewed also as biologic events related to focal adhesion. During the past decade, the functional role of focal adhesion–associated molecules has emerged in signal transduction. In this review, we use an operative definition of focal adhesion: a specific, localized, and transitory binding between a cell, often motile, and ECM or cell adhesion molecules. This binding implies that there are transmembrane molecules able to generate intracellular signals and cytoskeleton remodeling. In the past few years, certain protein tyrosine kinases (PTKs) have been identified as focal adhesion–specific kinases. This is particularly the case for the ubiquitous 125-kDa focal adhesion kinase ($p125^{FAK}$) that is involved in many important physiologic processes.

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Molecular Components

Several molecules actively intervene in the process of focal adhesion: ECM proteins and polysaccharides, and also cell adhesion molecules, which often share a common sequence with the matricial ones; transmembrane molecules, named focal adhesion effectors, that are coupled to adaptors usually specific of focal adhesion; and cytoskeleton and cell activation proteins, which constitute the final common pathways.

Effectors of Focal Adhesion

ECM is composed of high-molecular-weight molecules including collagens (at least 13 subtypes described) and other molecules involved in specific interactions during focal adhesion. Among these, proteoglycans interact with specific receptors expressed by many cell types and also function as membrane-associated molecules. For example, the heparan-sulfate cluster differentiation (CD) antigen 44 is involved in the homing of T lymphocytes into the thymus *(2)*; it is also expressed by B cells during their homing to germinal centers in lymph nodes *(3)*. Another molecule that intervenes in focal adhesion through binding to CD44 is hyaluronane, formed by up to 5000 disaccharides of the

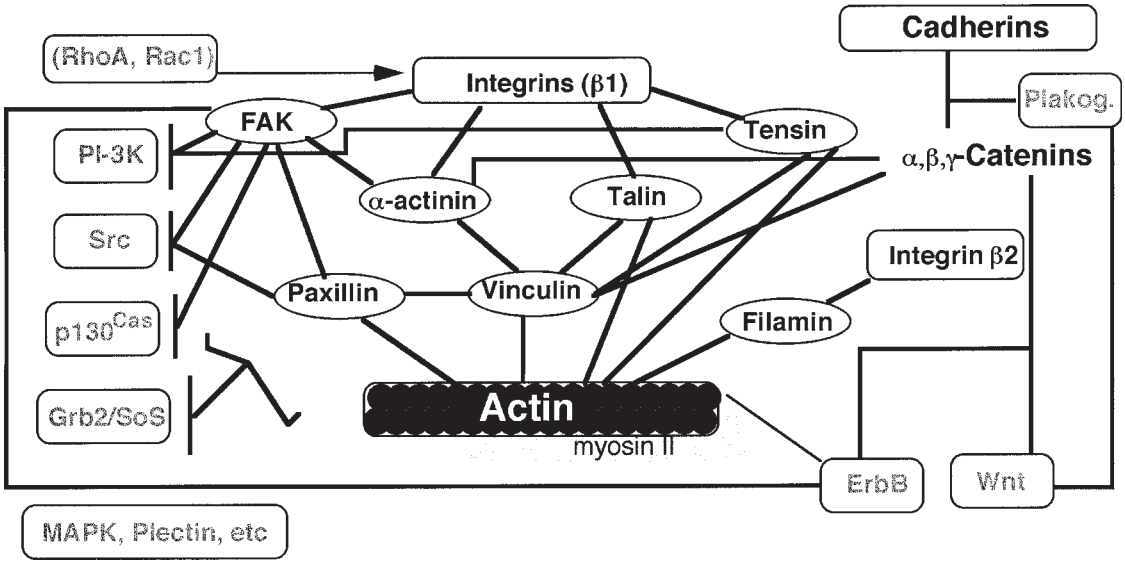

Fig. 1. Relationships of focal adhesion–related kinases with other molecules involved in the process of focal adhesion. Integrins of the β1 subtype, whose activation can be modulated by rho products, are known as activators of numerous focal adhesion–related molecules, including $p125^{FAK}$, α-actinin, tensin, and talin. Integrin β2 activates filamin. Among cell adhesion molecules, cadherins have been involved in focal adhesion through catenins. Paxillin and vinculin mediate many actions on actin, and a direct effect of filamin has also been described. Intracellular pathways (i.e., PI3-K, Src, $p130^{Cas}$-Crk, and Grb2/SoS) are activated after $p125^{FAK}$ phosphorylation. Interactions with differentiative pathways and homeotic genes also exist, such as $p125^{FAK}$ with ErbB, and catenins with ErbB and *Wnt*.

glucuronate subtype. The ECM component fibronectin is a heterodimer, and the 20 subtypes known to date are issued from a single gene by alternative splicing. Fibronectin can bind to collagen, heparan sulfates, DNA, and integrins and is a primary actor in focal adhesion *(4)*. Laminin is formed by three polypeptides and its total weight is 850,000. Among other molecules, laminin is able to bind to some collagens, proteoglycans, and specific surface receptors.

Adhesion molecules are generally named X-CAM (with X indicating the cell type). Many are classified in the CD system. A part of their sequence is homologous to ECM molecules, especially to fibronectin, and this homology renders them able to bind to integrins. Integrins are the most important primary effectors involved in focal adhesion. They are glycosylated heterodimers whose specificity for a ligand implicates arginine-glycine-aspartate residues (RGD), or lysine-aspartate-valine residues (LDV). For example, fibronectins bind via RGD or LDV sequences, whereas V-CAM only binds via an LVD sequence. An α- and a β-chain compose integrin heterodimers. Today, at least 8 β-chains and 16 α-chains have been identified, but all potential combinations are not encountered (to date, 22 combinations have been reported) *(5)*. Because no enzymatic activity is associated with integrins, adaptors and intermediate proteins mediate their properties in intracellular signaling.

Several molecules are connected to the effectors and modulate their activities. Members of the tetraspan superfamily are able to bind to the integrins and to phosphorylate their α-chain. They are also able to activate the phosphatidylinositol-4 kinase and the protein kinase C (PKC) pathways. For example, CD9, CD53, CD63, CD81, and CD82 expressed by B-lymphocytes are part of this family *(6)*. Syndecans have recently been identified as important molecules whose expression is regulated according to the cell type and the developmental stage. Their polysaccharidic part binds to a variety of molecules, and they control the relative density as well as the affinity of syndecan-binding surface molecules *(7,8)*. Other immunoglobulin (Ig) superfamily-related members (such as CD47 and CD147) are also implicated as integrin-associated molecules.

A wide range of membrane receptors also act on focal adhesion effectors, either directly or by specific interactions with intermediate molecules. This is the case for the T cell receptor (TCR) for antigen, receptors for cytokines (interleukin-2 [IL-2], IL-7) and growth factors (such as epidermal growth factor [EGF], platelet-derived growth factor [PDGF], transforming growth factor-β [TGF-β]), and the superfamily of G-coupled receptors with seven transmembrane domains.

Focal Adhesion–Related Kinases

Focal adhesion promotes a number of intracellular activation events. The focal adhesion–related 125-kDa kinase ($p125^{FAK}$) constitutes a crucial meeting point for many extracellular signals as different as integrin-binding molecules, mitogenic peptides (such as bombesin, endothelin, or vasopressin), TCR engagement, and oncogenic transformation. The phosphorylation of $p125^{FAK}$ is associated with important cellular events such as growth, motility, morphogenesis, and adhesion *(9)*. Other proteins directly involved in the focal adhesion trans-

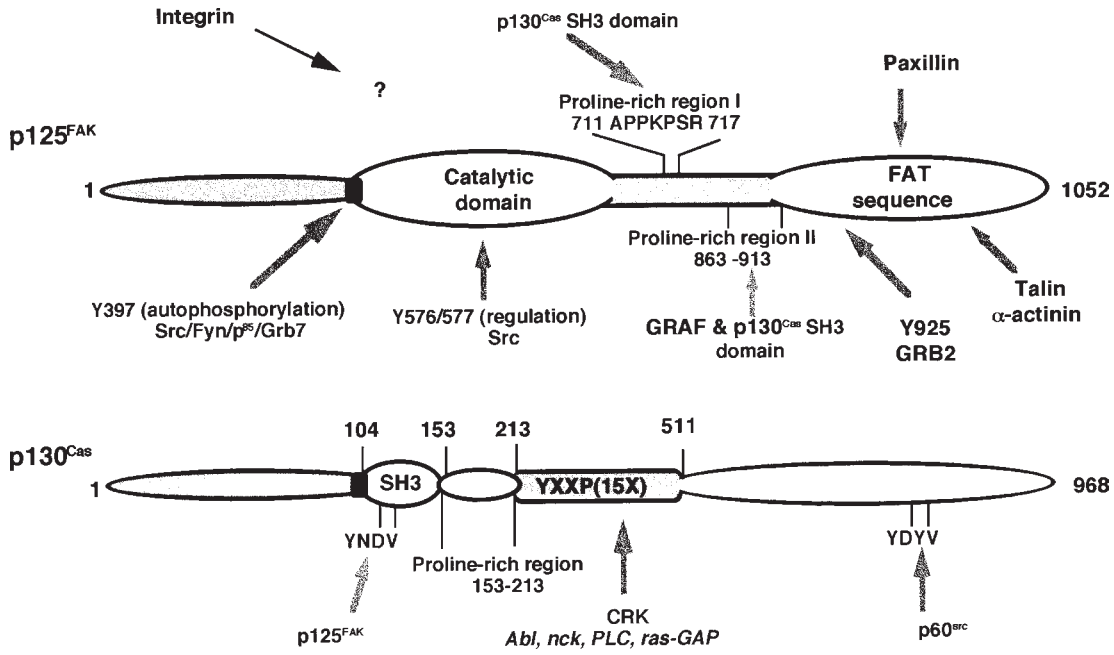

Fig. 2. Schematic representation of $p125^{FAK}$ and $p130^{Cas}$. The principal binding sites for other components of focal adhesion are indicated. Italics = putative proteins that could target to a site. Most of the data were taken from refs. *15* and *36*.

ducing system are paxillin, catenin, talin, vinculin, tensin, and actinin (Fig. 1). Whereas vinculin is usually involved in cell focal adhesion to matrix, catenin is more specifically associated with cell-to-cell signaling. Paxillin is a cytoskeletal protein that binds to actin. The small guanosine 5'-triphosphate (GTP)-binding proteins rhoA and rac1 directly act on integrins and are activated by growth factors *(10)*. In particular, $p21^{rhoa}$ is thought to mediate bombesin- and PDGF-stimulated focal adhesion to actin stress fibers *(11)*.

$P125^{FAK}$ (Fig. 2) is a member of a new family of nonreceptor PTK implicated in focal adhesion, morphogenic, and proliferative processes *(12)*. Unlike other nonreceptor PTKs, $p125^{FAK}$ does not possess any domain able to bind to membranes or other cellular proteins, the *src* homology-2 (SH-2) or SH-3 domains, and it does not include any consensus acylation site *(13)*. The C-terminal region is critical for efficient targeting to focal adhesion sites *(14)* and contains a sequence called focal adhesion targeting (FAT) that binds to $p130^{Cas}$, so that $p130^{Cas}$ can function as a downstream early signaling event *(15,16)*. The FAT sequence also binds to paxillin and can act through the adaptor proteins Grb2/SoS to activate the Ras/mitogen-activated protein kinase (MAPK) pathway. Grb7, an SH-2-containing and pleckstrin-homology domain-containing molecule, can be directly activated by $p125^{FAK}$, and could play a role in cell migration *(17)*. The numerous sites of tyrosine phosphorylation of $p125^{FAK}$ (Y-397, Y-407, Y-576, Y-577, Y-861, Y-925) mediate specific interactions with some docking proteins, together with the regulation of their own $p125^{FAK}$ kinase activity *(18–20)*. Since its discovery, the $p125^{FAK}$ family has grown and homologous proteins have been identified such as FakB (described in T cells) *(21–23)* and Pyk2/RAFTK/CAKβ/CadTK (first found in the neural cells) *(24)*. Pyk2 appears to be as ubiquitous as $p125^{FAK}$ *(25–28)*. The C-terminal domain of $p125^{FAK}$ itself, including the FAT sequence, is expressed autonomously as a 41-kDa protein (FAK-related nonkinase [FRNK]), whose expression is differentially regulated and which acts as a regulatory molecule without intrinsic kinase activity *(29,30)*.

Another protein involved as a transducing signal in focal adhesion is $p130^{Cas}$ (Fig. 2). $p130^{Cas}$ is phosphorylated in *src*-transformed fibroblasts and forms stable complexes with activated forms of p60*v-src* *(31–35)*. This protein contains an amino-terminal SH-3 domain and 15 sites of tyrosine phosphorylation. Each of these sites is positioned within the motif YXXP *(36)*, which can be bound by several proteins with an SH-2 domain *(37,38)*. The C-terminal region of $p130^{Cas}$ contains another putative SH-2 binding site that conforms to pp60*v-src* requirements *(37,38)*. The FAT sequence of $p125^{FAK}$ is a docking site for $p130^{Cas}$. This latter can associate in vitro and in vivo with $p125^{FAK}$ and function as a mediator in transduction of adhesion signals *(39,40)*. $p130^{Cas}$ is phosphorylated by vasopressin in cultured fibroblasts *(41)*. Paxillin and $p130^{Cas}$ phosphorylations could be regulated by $p125^{FAK}$ because they colocalize in cell focal adhesion, are physically associated with $p125^{FAK}$, and are coordinately phosphorylated on tyrosine residues by multiple stimuli *(12)*. The direct phosphorylation of $p130^{Cas}$ by $p125^{FAK}$ was recently demonstrated *(42,43)*.

Other putative substrates of $p125^{FAK}$ are paxillin and tensin. The 68-kDa paxillin interacts with many proteins known to intervene in cell transformation *(44)* and is phosphorylated on Y118 by $p125^{FAK}$ *(45)*. Tensin is an actin-binding protein, and its phosphorylation is important for the linkage of integrins to the actin cytoskeleton *(46)*.

Many interactions of membrane molecules with intracellular intermediate signaling proteins have been described (Fig. 1). The integrin β1 subunit can interact with talin, α-actinin, and $p125^{FAK}$ *(10,47)*. Integrin β1 clustering induces $p125^{FAK}$ phosphorylation and tensin aggregation on the cytoplasmic side of the membrane, together with other signaling molecules *(48,49)*. The interactions of integrin β2 with filamin *(50)* and of integrin α-subunit with actin and calcireticulin have also been reported *(51)*. It appears that only occupied integrins are able to fruitfully interact with cytoskeletal molecules (*see* review in ref. *52*). The integrin β4 subunit is tyrosine phosphorylated after binding, and its short cytoplasmic tail is a tyrosine-based activation motif analogous to T and B cell motifs *(53)*.

The adaptor proteins Shc and Grb2 can bind directly to the β domain of integrins and serve as intermediates toward the MAPK pathway *(53,54)*. The PDGF-mediated effect on $p125^{FAK}$ phosphorylation is interesting and remains to be explained further. At low doses, PDGF increases $p125^{FAK}$ phosphorylation, whereas, at higher doses (30 ng/mL), PDGF not only decreases it, but also inhibits bombesin-induced phosphorylation of $p125^{FAK}$, $p130^{Cas}$, and paxillin. This bell-shaped response curve could reflect a dissociation of actin stress fibers *(55)*. The phosphorylation of $p125^{FAK}$ and other focal adhesion–related kinases in chicken embryo cells is followed by the activation of the insulin receptor substrate-1 pathway, which leads to the phosphorylation of phosphatidylinositol-3 kinase (PI-3K) *(56)*. The GTPase regulator associated with focal adhesion kinase mediates cytoskeletal changes induced by rho *(57)*. Whereas the src-family tyrosine kinases Src, Fyn, and Yes effectively contribute to $p125^{FAK}$ phosphorylation; the mice deleted for these genes, however, show normal $p125^{FAK}$ phosphorylation and focal adhesion, although $p130^{Cas}$ is affected *(58)*. An explanation could be the dissociation of $p125^{FAK}$/$p130^{Cas}$/c-src complex that occurs during mitosis *(59)*. The dissociation between $p125^{FAK}$ and $p130^{Cas}$ kinase activity during some cell events indicates that these molecules can be regulated in different ways according to the cell-cycle phase. As another example of dissociation between $p125^{FAK}$ and $p130^{Cas}$, $p125^{FAK}$ activation is impaired by *Clostridium botulinum* C3 exoenzyme, which specifically inactivates rho, whereas no effect is observed on $p130^{Cas}$ and paxillin after stimulation with glial cell line-derived neurotropic factor (GNDF) *(60)*. The phosphorylation process and its time dependence appear to be of primary importance because focal adhesion is inhibited by the tyrosine kinase inhibitor herbimycin *(61)*, as well as by the phosphatase inhibitor orthovanadate *(62)*. The $p125^{FAK}$ homologous protein Pyk2 *(13,63,64)* is phosphorylated by tumor necrosis factor-α (TNF-α) in human promyelocytic leukemia cells (HL-60) *(65)*. Overexpression of Pyk2 leads to an increase in c-jun N-terminal kinase. PKC also is implicated in focal adhesion and is able to bypass $p125^{FAK}$ via a direct serine phosphorylation of paxillin *(66)*. During programmed cell death, capsases have been shown to interact with $p125^{FAK}$. Apoptosis-related caspases cleave the native kinases and generate an FRNK-like molecule that localizes in focal adhesion sites and serves as a dominant negative receptor for signaling by these kinases *(67)*. The same regulatory event appeared with $p125^{FAK}$-related protein Pyk2 with splice variants named PRNK *(68)*.

Thus, focal adhesion can be viewed as a general "booster" phenomenon that polarizes target cells and regulates their growth by contact inhibition, further differentiation, or transformation. In addition, the engagement of integrins is a key regulator of programmed cell death and cell cycle through cyclin-dependent kinase modulation.

Focal Adhesion in Immunoendocrinology (Fig. 3)

Because most immunocompetent cells are circulating elements, focal adhesion is an important biologic event in the development of an efficient immune response. For example, focal adhesion is essential for establishing close functional interactions between antigen-presenting cells and CD4/CD8 T lymphocytes in the initiation of the specific immune response, as well as between CD8 T cells and their target.

Thymic T Cell Homing and Differentiation

The homing of T lymphocyte progenitors from the bone marrow into the thymus is conditioned by CD44 expression. Other molecules, such as vanin-1 and GPI-80, are involved in pre-T cell homing and transendothelial migration *(69)*. Even if the formation of the trimolecular complex TCR/major histocompatibility complex (MHC)-antigen is not focal adhesion *per se*, the transient adhesion stabilizing this interaction deserves some interest in the general framework of this review. Within the thymic environment, pre-T lymphocytes are exposed to positive and negative selection. Under pre-T cell influence, TECs are induced to express specific adhesion molecules *(70)*. Pre-T cells also show a regulated expression of adhesion molecules, such as N-CAM *(71)*. During murine ontogeny, V-CAM is selectively expressed by cortical TECs and binds to pre-T cell integrin α4 β1 (VLA-4), whereas fibronectin, an alternative ligand for the same integrin, is selectively expressed by medullary TECs *(72)*.

In immunology, a remarkable observation is that a similar, but not identical, molecular interaction (TCR/MHC-antigen) leads to activation and proliferation of competent T cells in the periphery, whereas it induces either positive or negative selection of T cell clones in the thymus. Thymus differentiation of T lymphocytes involves focal adhe-

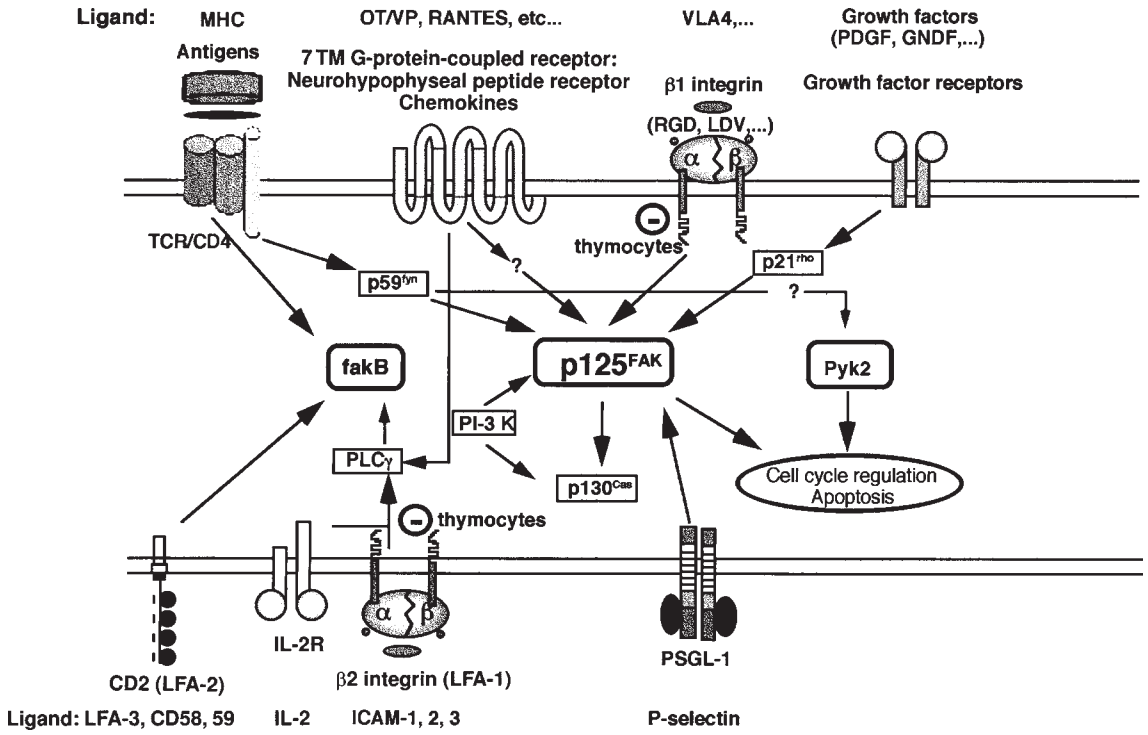

Fig. 3. Converging pathways in the phosphorylation of T cell focal adhesion kinases. Arrows indicate effective interactions. Some factors exert negative effects on thymic pre-T cells, but positive actions on peripheral mature T cells. Numerous pathways lead to the modulation of $p125^{FAK}$-related kinases. Interaction of TCR with the MHC-antigen complex phosphorylates $p125^{FAK}$ and Pyk2 (via $p59^{fyn}$) and directly activates fakB. The binding of seven transmembrane (7 TM) G-protein-coupled receptors to their natural ligands also activates $p125^{FAK}$, whereas β1 and β2 integrin engagement has the opposite effect depending on the maturation state of target T cells. Ig-like superfamily-related proteins, cytokine receptors (such as IL-2R), growth factor receptors, and P-selectin glycoprotein ligand-1 (PSGL-1) engagement have been shown to activate either fakB or $p125^{FAK}$.

sion molecules: whereas a high-affinity interaction of TCR with the MHC/self-antigen induces anergy or deletion, T cell activation can follow B7 (CD80)-CD28 interaction *(73)*. Adhesion molecules, such as integrin or VCAM-1, induce T lymphocytes to express CTLA-4, another receptor for B7 *(74)*. The active implication of $p125^{FAK}$ in thymic T cell differentiation was suggested by the presence of $p125^{FAK}$ mRNA in human T cells *(75)*, as well as in murine thymocytes (pre-T cells) at different differentiation stages *(76)*. Within T lymphocytes, $p125^{FAK}$ is activated by α4 β1 VLA-4 integrin engagement *(77)*. Because VLA-4 is activated by V-CAM and fibronectin found in cortical or medulla TECs, this could argue for the involvement of focal adhesion in thymic T cell differentiation. The $p125^{FAK}$-related protein fakB in T cells is phosphorylated following TCR engagement *(21)*, and this is enhanced by CD2 and CD4 costimulation. In addition, fakB can be phosphorylated via phospholipase Cγ (PLCγ) with no requirement for TCR engagement; this might indicate a link with the β2 integrin LFA-1 transducing system and with the G-protein-related receptor *(23)*. $p125^{FAK}$ itself is synergistically stimulated by integrins and TCR engagement in $CD4^+$ T cell blasts and Jurkat T-cells *(78,79)*. Pyk2 also is readily phosphorylated following TCR engagement *(80)*.

The thymic parenchyme expresses a repertoire of neuroendocrine-related precursors *(81)*. A hierarchy appears in the organization of this repertoire, because one dominant member in the thymus network represents each neuroendocrine hormone family. Regarding the neurohypophyseal hormone family, TECs and thymic nurse cells (TNCs) from various species including humans *(82)* dominantly synthesize oxytocin. Using immunocytochemistry and electron microscopy, investigators have observed numerous points of focal adhesion between oxytocin-synthesizing TECs/TNCs and pre-T cells (thymocytes). This was a strong morphologic argument supporting $p125^{FAK}$ implication in T cell development. Specific and functional neurohypophyseal receptors (oxytocin and V1 subtypes) are expressed by immature and cytotoxic T cells *(83)*. Neurohypophyseal hormones induce phosphoinositide turnover and $p125^{FAK}$ phosphorylation in RL-12NP cells, a murine pre-T cell line *(84)*. Regarding the kinetics of oxytocin-mediated $p125^{FAK}$ phosphorylation, the transient maximum peak coincided with that reported for T cell activation by the chemokine RANTES *(85)*. TCR engagement or integrin activation induces a more sustained phosphorylation of $p125^{FAK}$ *(77–79,86)*. A 130-kDa protein was also immunoprecipitated with anti-$p125^{FAK}$ detected in murine pre-T cells. The phospho-

rylation of this 130-kDa protein increased together with $p125^{FAK}$ after neurohypophyseal peptide stimulation, and most probably it corresponds to $p130^{Cas}$ *(84)*.

Both developmental arrest and apoptosis play an important role in thymic negative selection of self-reactive T cell clones. *(87)*. In human tumors, a relationship exists between the inhibition of $p125^{FAK}$ expression and the induction of apoptosis *(88)*. Our observations support this hypothesis but, overall, provide a molecular explanation to the morphologic data *(89)*. Induction of pre-T cell proliferation and focal adhesion–related kinase phosphorylation by hormones encountered within the thymic environment is in accordance with the model of cryptocrine signaling between TECs/TNCs and developing T lymphocytes *(81,90,91)*. The partial blockade of the $p125^{FAK}$ pathway in fyn-deficient and $p125^{FAK}$-heterozygous mice induces an impairment of thymocyte development at the double-negative $CD4^-CD8^-$ stage, which is the earliest developmental stage in the thymus *(92)*. From these independent observations, it clearly appears that the phosphorylation of focal adhesion–related kinases induced in vivo by natural thymic peptide ligands deserves to be investigated further as an important pathway of T cell differentiation. This conclusion is reinforced by the recent finding that gene deletion of PI-3Kγ induced a marked impairment of T cell development and activation *(93)*.

Focal Adhesion and Inflammation

Leukocyte infiltration during inflammation is one of the best examples of regulated adhesion. The mechanism called rolling is divided into three phases: targeting, adhesion, and leukocyte extravasation. The preliminary tropism of leukocytes involves several chemokines. It is followed by a binding of L-selectin expressed by lymphocytes to Gly-CAM-1 and glycosylated CD34 on endothelial cell, and this permits the adhesion process and rolling itself. Chemokines bound to ECM then promote lymphocyte β2 integrin LFA-1 activation. Activated LFA-1 binds tightly to ICAM-1, and this interaction allows diapedesis and extravasation of leukocytes. Many other adhesive molecules intervene in these processes *(94–96)* and can be related to focal adhesion itself, as well as to other inflammatory events such as protease activation or oxygen liberation.

Adhesion molecules in inflammation have been repeatedly reviewed in recent years *(97–100)*, but little is known about the implication of focal adhesion kinases in inflammation and inflammatory diseases. Nevertheless, the proinflammatory IL-1β induces a decrease in the level of $p125^{FAK}$ in an endothelial cell line *(101)*, whereas T cell adhesion to P-selectin increases $p125^{FAK}$ phosphorylation *(86)*. These observations indicate that focal adhesion-related kinases could intervene in the control of inflammation.

Focal Adhesion in Developmental Biology

Two key biologic processes during embryonic development are cell migration and cell-to-cell adhesion. Thus, the molecular bases of focal adhesion are important to the understanding of how embryonic cells recognize and interact with their neighbors. Homeotic genes play an orchestrating role in the program of embryonic differentiation and activate many other genes implicated in the spatiotemporal regulation of the expression of adhesion- and motility-related molecules *(102–105)*.

Early events in ontogeny are blastocyst implantation and acquisition of an invasive phenotype by embryo trophoblastic cells. Trophoblastic cells express adhesion molecules in a spatially regulated pathway and induce modification in the surrounding decidualized stromal endometrium. Cytotrophoblastic cells show a reduced staining for adhesion molecules characteristic of polarized cytotrophoblastic epithelial stem cells, such as integrin α6 β4 and E-cadherin, and display a panel of adhesion receptors characteristic of endothelium. From the embryonic to the maternal side, cells are stained for vascular/endothelial-cadherin, VCAM-1, platelet/endothelial-CAM-1, αVβ3, and α1β1 integrins. Interestingly, cytotrophoblastic cells are stained for a melanoma-associated IgG family receptor (Mel-CAM) also expressed by endothelial cells in different organs *(106)*. The tumor-like invasive phenotype of trophoblastic cells is regulated by the nature of integrins engaged *(107)*. Finally, the cytotrophoblastic cells within the maternal blood vessels are positive for N-CAM expression. Matrix metalloproteases and their inhibitors can be specifically induced by the invasive trophoblast *(108,109)*, e.g., metalloproteinase-9 *(110)*. This indicates that embryonic cells not only adhere to maternal ECM but also remodel it in a specific manner.

$p125^{FAK}$ is crucial in early embryonic development, as demonstrated by the mesodermal defect during gastrulation of $p125^{FAK}$-deleted mice *(111–113)*. The lethality of the embryo seems to be due to a dramatically reduced motility of mesodermal cells *(112)*. The absence of a change in growth rate in $p125^{FAK}$-deficient embryonic stem cells *(114)* suggests that the mesodermal defect results from the absence of cell migration and differentiation induced by $p125^{FAK}$. The *Armadillo* homolog β-catenin is connected to the Erb pathway (including EGF receptor) and to the *Wnt* pathway, and induces the dorsal mesoderm in *Xenopus* through lymphoid enhancer-binding factor-1 *(115)*. This suggests an intimate relationship between focal adhesion kinases and homeotic genes. Recently, the contribution of focal adhesion kinases to developmental biology and cell life cycle was reinforced by the demonstration that mitosis modulates the way in which $p125^{FAK}$ and $p130^{Cas}$ are coordinately phosphorylated *(59)*. Also,

Table 1
Major Signaling Pathways Associated with Focal Adhesion

Ligand	Receptor	Transduction	Focal adhesion component
MHC/antigen	TCR	$p59^{fyn}$	$p125^{FAK}$, Pyk 2
ECM components (fibronectin, vitronectin, laminin, collagen)	Integrins	PKC involvement	$p125^{FAK}$, $p130^{Cas}$
VLA-4	β1	PLCγ	α-Actinin, $p125^{FAK}$, tensin, talin
ICAM-1, -2, -3	β2		Filamin, fakB
Collagen	TM4SF (integrin-associated)		$p125^{FAK}$
LFA-3, CD58, CD59	Ig superfamily (CD2)		fakB
Peptide hormones	G-coupled 7 transmembrane	PLCγ	FakB
		? (*see* Fig. 3)	$p125^{FAK}$
Growth factors EGF, PDGF, GNDF, TGF-β	Growth factor R	$p21^{rho}$	$p125^{FAK}$
Cytokines			
IL-2	IL-2R (β2-integrin associated)		FakB
TNF-α	TNF-α RII		Pyk2
Cadherin	Cadherin	α-, β-, γ-catenins	α-Actinin, vinculin
P-Selectin	PSGL-1		FakB, $p125^{FAK}$

[a]References are given in the text

$p125^{FAK}$ interacts with Grb7 *(17)*, a protein related to *Caenorhabditis elegans* gene *mig-10*, which is involved in the migration of embryonic neurons *(116)*. $p130^{Cas}$ has a structure similar to embryo fyn-associated substrate *(117)*. However, our knowledge about the precise roles of focal adhesion kinases in embryonic development is restricted by the early embryo lethality (d 8.5 in mice) due to the mesodermal defect in the late phase of gastrulation. It is expected that the creation of conditional defective mutants could provide some answers to that problem.

Focal Adhesion and Cancer

The important implication of $p125^{FAK}$ in oncogenic transformation has been demonstrated by many studies *(118–123)*. $p125^{FAK}$ was initially evidenced in v-*src*-transformed chicken cells, and $p125^{FAK}$ overexpression is directly implicated in the metastatic power of prostate, colon, and breast cancer *(119,124)*. The invasive phenotype of cancer cells has been proposed to be controlled in part by 125^{FAK} *(119,124)*, and $p125^{FAK}$ is highly phosphorylated in leukemic cells expressing $p210^{BCR\text{-}ABL}$ *(125)*. The binding of integrins to their ECM ligands induced by $p125^{FAK}$ and the secondary cell spreading may represent critical steps for a normal cell anchorage *(126)*. The controversy about the role of $p125^{FAK}$ either in activation or in inhibition of tumor cell spreading can be resolved by proposing a $p125^{FAK}$ alternative pathway to Grb2 or MAPK signaling. According to that proposition, $p125^{FAK}$ phosphorylation activates proliferation that could be overboosted by oncogenic stimulation of $p125^{FAK}$ itself *(127)*, and various oncogenic proteins could lead to a shortcut so that integrin-mediated $p125^{FAK}$ activation would no longer be needed. The oncogenic adaptor protein v-Crk may function similarly to $p125^{FAK}$, increasing $p130^{Cas}$ and its own dowstream pathways of stimulation *(128,129)*. The oncogenic variants of Src also increase $p125^{FAK}$ and $p130^{Cas}$ phosphorylation *(127)*. Finally, $p125^{FAK}$ antisense reduces adhesion and induces apoptosis in tumor cells with a minimal effect on normal cells *(130)*. Altogether, these observations demonstrate that $p125^{FAK}$ phosphorylation plays an important role in cancer and represents an important molecular target for novel anticancer therapeutic strategies.

Conclusion

Focal adhesion constitutes a new fertile domain for research in cell biology and signal transduction. Its implication in many physiologic events is just emerging, but already suggests innovative therapeutics (i.e., $p125^{FAK}$ antisense against tumor cells with limited effects on normal cells). The knowledge of their precise role and functional activity requires the acquisition of an integrative viewpoint onto their multiple pathways of activation and

downstream relays to the cytoskeleton and intracellular events. Obviously, the complexity of a shortcut and the multiplicity of molecular components sharing common actions is hard to handle (Table 1). Nevertheless, the use of specific inhibitors and phosphorylation site-directed antibodies will be of great help to understand the precise involvement of the adhesion kinase alphabet and syntax in signal transduction and cell communication.

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