**[Frontiers in Bioscience 3, d877-886, August 5, 1998]**

# CONSERVED FUNCTIONS OF THE CYTOPLASMIC DOMAINS OF INTEGRIN BETA SUBUNITS

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Received 6/14/98 Accepted 6/22/98

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## 1. ABSTRACT

In this article I will discuss the notion that many integrins have similar functions in cell spreading, organization of the cytoskeleton and intracellular signalling. Most of these functions are transmitted through the cytoplasmic domains of integrin beta-subunits. These parts are also quite conserved between most integrins. I will discuss, what is known about the molecules binding to these parts of integrins, and which of those transmit the conserved functions.

## 2. INTRODUCTION

The integrins were so named because they integrate or link extracellular matrix structures to cytoskeleton (1). Some integrins also work in cell-cell adhesion (2). Integrins are a superfamily of dimeric proteins composed of alpha and beta subunits. Generally, homology between different alpha subunits or beta subunits is less than 40%, but each subunit has conserved structural features.

In addition to the conserved structures, integrins share several functional features. Figure 1 summarizes the functional cycle of many (if not all) integrins. They can exist in at least three conformational forms in the plasma membrane: resting, activated and ligand-bound. Apparently, both the extracellular parts and the short cytoplasmic tails of integrins can attain the three conformations. Interactions between the subunits are most probably involved in the transmission of conformation changes through the membrane. Movement in or out of the plane of membrane are also suggested to be involved (3). The cytoplasmic parts are involved in both the activation of integrins and in the post-ligand binding events: interaction with cytoskeleton, clustering and signal transduction.

This article focuses on the cytoplasmic domains of integrin beta subunits that are known to mediate the integrin-cytoskeleton linkage and are also involved in integrin activation and downstream signalling from integrins. Particularly, I will discuss the conserved cytoplasmic domain of the integrin subunits beta1A, beta1D, beta2, beta3, beta5, beta6, and beta7. I will discuss the conserved features of their structures, functional sites predicted by mutational studies, structural models, binding molecules and how these all are related to the conserved functions of these domains.

## 3. THE CLASSIFICATION OF THE CYTOPLASMIC DOMAINS OF INTEGRIN BETA SUBUNITS

Today, eight different integrin beta subunits are known. Several of these occur in variant forms generated by alternative splicing (4). The sequences of the cytoplasmic domains of all these integrins and their variants can be divided in four groups:

1. beta1-like cytoplasmic domains
2. truncations or aberrations of beta1-like cytoplasmic domains
3. beta4 cytoplasmic domain
4. beta8 cytoplasmic domain

The different cytoplasmic domains belonging to these groups are listed in table 1.

Based on sequence homology, seven integrin beta subunit cytoplasmic domains can be grouped together. I have

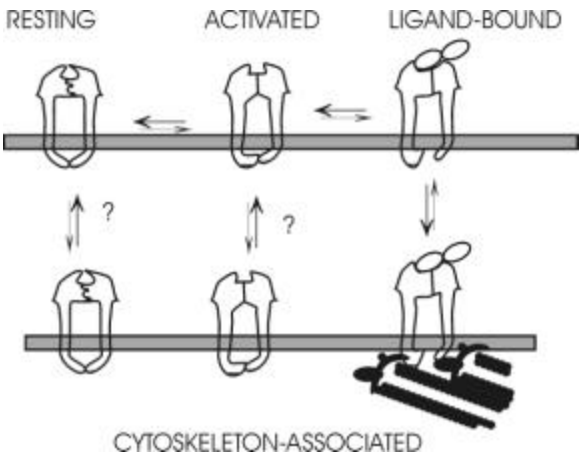


**Figure 1.** The functional cycle of integrins. Integrins can exist on the plasma membrane in at least three different conformations that can be distinguished by their different ligand- and cytoskeleton-binding properties and by some antibody epitopes. Each of these conformers may cluster differentially on the plane of membrane. The major conformed binding to the focal adhesion structures is the ligand-bound form.

**Table 1**. Grouping of the cytoplasmic domains of integrins

| | |
|---|---|
| **Beta1-like cytoplasmic domains** | beta1A (5; 6)<br>beta1D (7)<br>beta2 (8)<br>beta3 (9)<br>beta5 (10; 11)<br>beta6 (12)<br>beta7 (13; 14) |
| **Aberrations of beta1-like cytoplasmic domains** | beta1B (15)<br>beta1C (16)<br>beta3B (17) |
| **Beta4 cytoplasmic domains** | beta4(A) (18)<br>beta4B (19)<br>beta4C (20)<br>beta4D (21) |
| **Beta8 cytoplasmic domain** | beta8 (22) |

included beta1A, its muscle-specific splicing variant beta1D, beta2, beta3, beta5, beta6, and beta7 in this group. I will call this group the beta1-like cytoplasmic domains, because beta1A was the fist of them sequenced and still best studied. The beta1-like cytoplasmic domains are the most common cytoplasmic domains of integrins and they are quite homologous to each others. The beta1-like cytoplasmic domains link to actin cytoskeleton. They are the major focus of this review and are thus discussed further in the next chapters.

Aberrant splicing variants of the beta1-like cytoplasmic domains seem to devoid most of the functions of the more abundant, normal splicing products. An excellent review about their function has been published recently (4).

The integrin beta4 cytoplasmic domain is completely different in size and sequence from all the other beta subunits (18-20). It contains over 1000 amino acids, whereas all the other beta subunit cytoplasmic domains contain less than 60. The beta4 cytoplasmic domain mediates linkage to keratin type intermediate filaments in hemidesmosomes of epithelial cells (23). Thus, the beta4 cytoplasmic domain differs both functionally and structurally from the other integrins.

The beta8 integrin subunit forms a dimer with the alphaV subunit (22), which can also associate with the beta1, beta3, beta5, and beta6 subunits (24; 25). The alphaVbeta8 integrin seems to be functional in cell adhesion (22), but its functional relations to the other alphaV-integrins are not clear. The divergent sequence of the beta8 cytoplasmic domain suggests that it would have a different mode of function than the other integrin beta subunits.

## 4. THE PRIMARY STRUCTURES OF THE BETA1-LIKE CYTOPLASMIC DOMAINS

When the sequences of the beta1-like cytoplasmic domains of integrins are compared with each others, one can note that they share different degree of homology. If the beta-beta1D pair is not considered (because the alternative splicing site is close to the COOH-terminus), the most similar pair of these sequences are beta3 and beta6 , which are 67% identical and 77% similar. The most unrelated pair are beta2 and beta5 , which are 32 % identical and 54 % similar. The similarity of the cytoplasmic domains of these integrins is much higher than the overall similarity of the proteins.

When the cytoplasmic domains are aligned, one can note that the similarity between the sequences extend all along their length (figure 2). The most variant regions reside at the COOH-terminus, the beta5, beta6, and beta7 sequences being 7-11 amino acids longer that the beta 1A. The extensions locate mostly to the COOH-termini. The beta5 sequence can be aligned in two different ways with the others When the functionally important NXXY sequence (see below), close to the COOH-terminus, is aligned (figure 2), the beta5 cytoplasmic domain seems to have an 8-amino acid insertion right before this sequence. An alternative (figure 2B) alignment, without any large insertions, also aligns some conserved residues of the COOH-terminus of the beta5 with the others, and this alignment seems not to be quantitatively much different from the other alignment. Interestingly, some homological residues can be seen in this straight alignment between the COOH-terminal regions of beta5 and beta6.

Mutational scanning experiments of the cytoplasmic domain of beta1 have revealed three functionally important areas. In the original study each amino acid of the beta1 subunit cytoplasmic domain was individually mutated to alanine and the ability of these mutant to organize to focal adhesions was analysed (26). The three most conserved areas in the beta1 subunit cytoplasmic domain turned out to be the functional hot spots in this assay. These areas were named cyto-1, cyto-2 and cyto-3. The cyto-1 is located close to the transmembrane region, and characterized by acidic and aromatic amino acids $D^{764}$, $F^{768}$, $F^{771}$ and $E^{774}$. The cyto -2 in composed of the $N^{785}PIY^{788}$ sequence of beta1.

A:

| | |
|---|---|
| consensus | kllv.ihDrrEfakfekE...akwdt..NPlyksa..tf........kNp.y..k..... |
| **beta1A** | **KLL**MI**IHDRREFAKFEKE**KMN**AKWDT**GE**NPIYKSA**VTT**V**.....V**NP**KY**EG**K** |
| **beta1D** | **KLL**MI**IHDRRE**F**AKFEKE**KMN**AKWDT**QE**NPIYKS**PIN**NF**.....**KNP**NY**GRK** |
| **beta2** | **KA**L**I**HLS**D**L**RE**YRR**FEKE**KLKSQ**W**NN.D**NPLFKSA**TTT**V**.....M**NP**K**F**AES** |
| **beta3** | **KLL**IT**IHDR**K**EFAKFE**EE**RAR**AKWDT**AN**NPLYK**EA**TS**TF**.....T**N**IT**Y**R** |
| **beta5** | **KLL**VT**IHDRREFAK**.QS**E**RSR**A**RYEMAS**NPLY**RKPIS**T**HTVDFTFNKF**N**KS**Y**NGTVD** |
| **beta6** | **KLL**VSF**HDR**K**EVAKFE**AERSK**AKW**Q**T**GT**NPLY**RGSTS**TF**.....**KN**VT**Y**KHREJQKVDLSTDC** |
| **beta7** | **RL**SVE**I**Y**DRRE**YSR**FEKE**QQQLN**W**KQDS**NPLYKSA**ITT**T**.....I**NP**RFQEADSPTL** |
| **beta8** | **KVL**IIRQVILQWNSNKIKSSSDYRVSASKKDKLILQSVCTRAVTYRREKPEEIKFTMDISKLNAHETFRCNF |

B:

| | |
|---|---|
| consensus | kllv.ihDrrEfakfekE...akwdt..NPlyksa..tf.np.y.ek...k....T.. |
| **beta1A** | **KLL**MI**IHDRREFAKFEKE**KMN**AKWDT**GE**NPIYKSA**VTT**VV**NP**KY**EG**K** |
| **beta1D** | **KLL**MI**IHDRRE**F**AKFEKE**KMN**AKWDT**QE**NPIYKS**PIN**NF**K**NP**N**Y**GR**K** |
| **beta2** | **KA**L**I**HLS**D**L**RE**YRR**FEKE**KLKSQ**W**NN.D**NPLFKSA**TTT**V**M**NP**KFAES** |
| **beta3** | **KLL**IT**IHDR**K**EFAKFE**EE**RAR**AKWDT**AN**NPLYK**EA**TS**TF**T**N**IT**Y**R** |
| **beta5** | **KLL**VT**IHDRREFAK**.QS**E**RSR**A**RYEMAS**NPLY**RKPIS**T**HT.VDFTFNKFN**K**SYNG**T**VD** |
| **beta6** | **KLL**VSF**HDR**K**EVAKFE**AERSK**AKW**Q**T**GT**NPLY**RGSTS**TF**K**N**VT**Y**KHREJQ**K**VDLS**T**DC** |
| **beta7** | **RL**SVE**I**Y**DRRE**YSR**FEKE**QQQLN**W**KQDS**NPLYKSA**ITT**TIN**PRFQEADSPTL** |

**Figure 2.** Two different alignments of beta1-like integrin cytoplasmic domains (AB). The COOH -terminus of the subunit is shown on the right and the end of the transmembrane region of the left. The beta8 sequence (bottom row in A) has very little homology to the sequences in this group. The residues identical to the consensus sequence are shown in bold.

Within this sequence mutations at $N^{785}$, $P^{786}$ and $Y^{788}$ decrease integrin function whereas mutations of $I^{787}$ have no effect (26). Thus, this sequence is also called the NPXY motif. The cyto-3 is homologous to the cyto-2, having the sequence $N^{797}PKY^{800}$ in beta1. However, in the cyto-3 region only mutations at $N^{797}$ or $Y^{800}$ have a functional effect. Thus, this area is also called the NXXY motif. In this and several other studies (27-32), substitutions of the tyrosine residues at the NPXY or NXXY motifs with phenylalanine had no effect to the function, suggesting that it is rather structure of these motifs than phosphorylations of tyrosine that is functionally important. Recently, however, phosphorylation of beta3 to the NPXY tyrosine has been shown to be required for some of its functions (33).

Mutation studies have also been published on the beta2 and beta3 integrins (27-32; 34). Largely, the same functional regions have been located in the cytoplasmic domains of these integrins as in beta1. In addition, mutations at the region between the NPXY and NXXY motifs have been found to have functional consequences (27; 28; 30-32; 34). In beta2, substitution of the TTT sequence stretch with AAA abrogated cell adhesion (27), and in beta3 a human mutation have been found at this area leading to non-functional beta3 integrin subunit and to platelet aggregation deficiency, Glanzmann's thrombastenia (35). This mutation is S-P substitution at position 752 of beta3, which apparently leads to local misfolding of the proteins, since more conservative substitutions at the same position have less effects (30).

Depending on the functional assays used in the studies, quantitative differences have been observed between the effects of mutations targeted to the various functional areas of the beta1-like cytoplasmic domains. The most severe defects are caused by mutations in the NPXY motif. For instance, Y-A substitution mutant of the NPXY motif in beta3 totally lacks the ability to mediate cell spreading, whereas substitution at the membrane-proximal area (cyto-1) or the NXXY motif only partially inhibit this function (30). On the other hand, results with COOH-terminal deletion mutations suggest that some features of the NXXY motif and the amino-acid preceding it are also needed for cell spreading function of beta3 (30) (see further below).

## 5. HINTS OF THE 3-DIMENSIONAL STRUCTURES

Actual three dimensional structural data of the cytoplasmic domains of integrin beta subunit does not exist. However, mutation studies give some hints about the structures (26), circular dichroism spectra has been produced from synthetic peptides of beta3 (36; 37) and models for the 3-dimensional structure has been presented (37).

Much of our thinking about the folding of the beta1-like integrin cytoplasmic domains is based on the assumption that the conserved NPXY region would form a beta-turn structure. NMR studies have shown that NPXY sequences in insulin receptor and LDL receptor adopt a type 1 beta-turn conformation (38; 39). Interestingly, this motif has been identified as a signal needed for coated pit-mediated endocytosis of some receptors (40). There are some reports that NPXY as an endocytotic signal would not be in a beta-turn conformation (41). In mutational studies the importance of both proline and aromatic residues is an indication of the beta-turn conformation (26). On the other hand, substitution of the NPXY motif with another known beta-turn motif, YTRF, produces a non-functional integrin cytoplasmic domain (31; Ylänne *et al*., unpublished).

The Mutational studies suggest that the membrane-

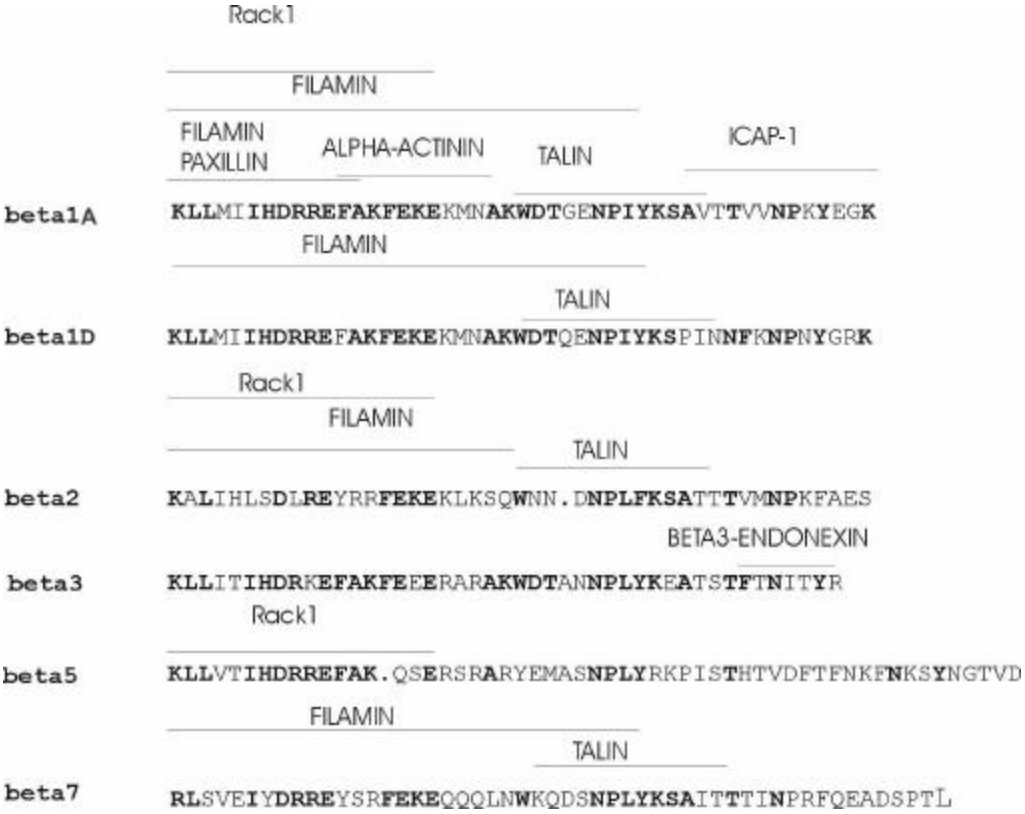


**Figure 3.** Known interaction sites in the beta1-like integrin cytoplasmic domain. References can be found in table 2.

proximal region of the beta1 cytoplasmic domain would attain an alpha-helical conformation (26). This was proposed, because the functionally sensitive residues could be located on one side in a helical wheel projection. Indeed, in certain conditions, high alpha-helicity can be observed in circular dichroism studies of beta3 cytoplasmic domain peptides (37). The alpha-helical region may also function as an inter-subunit interaction site (37; 42).

The 3-dimensional models of the cytoplasmic domain of the beta3 subunit are based on the beta-turn model of the NPXY motif (37). This model brings the membrane-proximal part and the COOH-terminal parts, including the NXXY motif, to close proximity. Interestingly, this might provide an explanation why some integrin-binding proteins appear to have a split binding site in the cytoplasmic domain (43).

Overall, based on sequence homology, most of the suggested features of the 3-dimensional structures of beta1-like cytoplasmic domain can easily be assigned to any of the proteins in the group. This is because the suggested structural elements are in the most conserved parts of the sequences. It would be of interest to model the less-homologous regions of beta5, beta6, and beta7 subunits, how these would fit in the proposed models. On the others hand, comparison of the functions of these regions by using mutagenesis approaches might also give some hints for the structural studies.

## 6. MOLECULES INTERACTING WITH THE BETA1-LIKE CYTOPLASMIC DOMAINS

Table 2 summarises the proteins know to interact with beta1-like integrin cytoplasmic domains. I have classified the binding proteins in two groups: (i) those binding to many or most beta1-like cytoplasmic domain, and (ii) integrin-specific beta subunit binding proteins

Figure 3 summarises the known interaction sites. Interestingly, many of the integrin-specific binding proteins bind to the COOH-terminal regions of the subunits, although the binding sites of all of these are not known yet. On the other hand, these integrin-specific binding proteins seem to be completely unrelated with each others. Unfortunately, very little is known of the proteins associated with the beta6, and beta7 cytoplasmic domain.

## 7. CONSERVED FUNCTIONS OF THE BETA1-LIKE CYTOPLASMIC DOMAINS

Above I have discussed the conserved structural features of the beta1-like cytoplasmic domains, and their

**Table 2.** Proteins interacting with the cytoplasmic domains of integrin beta-subunits

| | | |
|---|---|---|
| Proteins interacting with many integrin beta subunits | talin | beta1A, beta1D, beta7 (44-46) |
| | ABP280/filamin | beta1A, beta1D, beta2, beta7 (45;47) |
| | FAK | beta1A, beta3? (48; 49) |
| | paxillin | beta1A, beta3?(48) |
| | alpha-actinin | beta1A and others ?(43; 50) |
| | Rack1 | beta1A, beta2, beta5 (51) |
| | ILK | beta1A, beta3 (52) |
| Proteins interacting with a specific integrin beta subunit | ICAP-1 | beta1A (53) |
| | cytohesin-1 and homologs | beta2 (54) |
| | beta3-endonexin | beta3 (55; 56) |

known binding proteins. Here I shall discuss three conserved functions of these domains: (i) cytoskeletal anchorage and assembly of focal adhesions, (ii) transdominant inhibition of each others's functions, and (iii) signalling functions.

### 7.1. Cytoskeletal anchorage and assembly

All beta1-like integrin cytoplasmic domains mediate linkage to actin cytoskeleton. In the case of beta1A, beta2, and beta3, this functional feature seems to be interchangeable. When recombinant integrin beta1A subunits are made in which the cytoplasmic domain of beta1A has been swapped to that of beta3 (57), or beta3 to beta1A, or beta2 (30; 31; 34; 58), the ability of the chimeras to organize focal adhesions seems to be at least qualitatively retained. The corresponding swapping experiments have not been done with beta1D, beta5, beta6, or beta7, but at least beta1D, and beta5, and beta6 have been shown to be localized to focal adhesions. The ability of the beta5 subunit to localize to focal adhesions, however, is weaker than that of beta3 (59).

This suggests that the ability to induce focal adhesion assembly would be an important conserved function of the beta1-like integrin cytoplasmic domains. Apparently this is a very complex process involving reorganization of actin filaments and several signalling events. For instance, tyrosine kinase inhibitors have been shown to inhibit focal adhesion assembly and cell spreading (60). Also inositol phosphate metabolism, for instance P-Ins-5 kinases (61) and the P-Ins-3 kinase (62) might be involved. However, when various enzyme inhibitors are shown to inhibit focal adhesion assembly, we cannot be sure whether they affect some signals initiated by integrins or, more generally the equilibrium and dynamics of actin cytoskeleton needed for the response to these signals.

### 7.2. Trans-dominant inhibition of function

The other hint that certain functions of the integrin beta1-like cytoplasmic domains are conserved, comes from studies in which single cytoplasmic domains are introduced to cells by hooking them to some other, presumably irrelevant, transmembrane proteins. In such cases, overexpression of these single chain integrin cytoplasmic domain constructs leads to inhibition of the function of cellular integrins (63-66). For this dominant negative inhibition, cross-linking of the recombinant integrin chimera seems not to be needed, but overexpression is required. It has been shown that the beta1A and beta3 cytoplasmic domain inhibit the function of each others in these assays. Also the cytoplasmic domain of beta5 will inhibit the function of cellular beta1 and beta3 integrins, but less efficiently than those themselves (67; 68). This inhibition of function can be explained either by the ability of the chimera to compete effectively for a limited pool of cytoplasmic binding proteins or to saturate some signal transduction cascaded required for integrin function. In either case integrin-dependent cell adhesion and other functions will be abrogated.

The trans-dominant inhibition of integrin function is not only the property of single chain integrin beta subunit cytoplasmic domain chimeras. Inhibition can also be observed between different integrin dimers. For instance, binding of specific peptide ligands to the alphaIIbbeta3 integrin inhibits the function of alpha2beta1 and alpha5beta1 integrins (69).

### 7.3. Signalling functions

Integrin-dependent intracellular signals are a major research focus in the field. As signal transduction in general, also integrin-dependent signals are a complicated meshwork of parallel reactions that are dependent on each others. The outcome of signalling events is also dependent on the differentiation and proliferation status of the given cell or cell line.

Integrin-dependent signals can be classified to at least three categories: (i) Signals by associated transmembrane molecules (Growth factor receptors, TM4-family of proteins, IAP/CD47, caveolin), (ii) direct signals to classical growth factor pathways via integrin-associated cytoplasmic proteins (FAK, Sch, Grb2), and (iii) other signals through novel proteins to less-characterized pathways. The integrin-associated signals have been extensively covered in several recent reviews (70-75). Here I will only discuss some features of the above categories of signals that are relevant to the focus of the article.

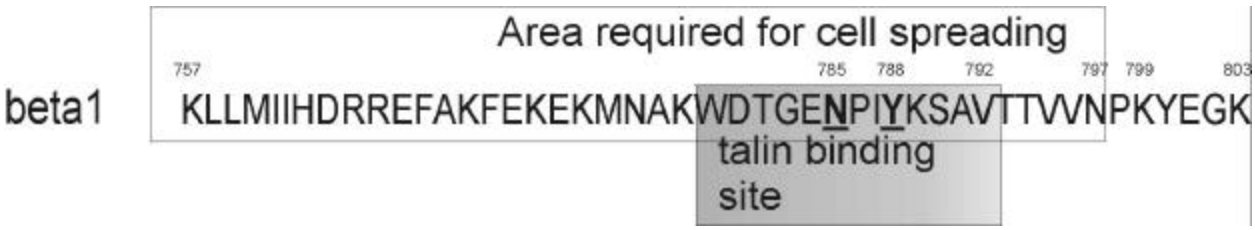


**Figure 4.** Summary of our studies, in which various point mutations and deletion mutations of the integrin beta1A subunit were studies for their ability to bind to talin (beta1A mutants expressed as GST fusion proteins in E. Coli) and for their ability to mediate cell spreading, when expressed as a chimeras with the extracellular and transmembrane domains of beta3 subunit (Kääpä, Peter and Ylänne, submitted). Point mutations at the underlined amino-acid blocked both talin binding and cell spreading whereas deletion mutation studies revealed different requirements for talin-binding and cell spreading in these models.

The association of growth factor receptors to integrin clusters can be mediated through the cytoskeletal organization induced by integrins. It is known that several growth factor receptor-associated molecules interact with cytoskeleton. On the other hand, the finding that caveolin associates with integrins (76) rises the possibility that certain integrins (in certain conditions) may localize to some membrane lipid domain. Caveolin has been shown to be associated with cholesterol- and glycosphingolipid-rich phospholipid rafts (77-79), which may also attract some signalling receptors. Aside from the co-immonoprecipitation studies, other data of integrin association with such domains is mostly lacking, however. One complication may be that the different conformational forms of integrins (figure 1) may have different preference to these membrane domains, and it may be difficult or impossible to purify the specific conformers. Whether integrin association to certain membrane domains and growth factor receptors is a conserved feature between different integrins, remains to be established.

On the other hand, the association of integrins with other transmembrane proteins such as the integrin-associated protein (IAP)/CD47, or proteins of the four-transmembrane domain (TM4 or tertraspan) family seem to be integrin-specific (80-82). These associations have a role in integrin-specific signal transduction.

In addition to contributing to growth factor signalling by localizing growth factor receptor to cell contact areas, integrins also directly modulate growth factor signalling via focal adhesion-associated proteins. The focal adhesion kinase (FAK) may have an important role in this respects. However, recently it was shown that FAK is not needed for the assembly of focal adhesions (83) and that integrins can activate the MAP-kinase pathway independent of FAK (84). Aside FAK, many focal adhesion proteins such as paxillin, p130Cas, tensin, zyxin and VASP can mediate link to the components of MAP kinase pathways. Some of these links may be a common function between the beta1-like integrin cytoplasmic domains.

## 8. CORRELATION BETWEEN COMMON BINDING PROTEINS AND COMMON FUNCTION

Talin, alpha-actinin and filamin are the current major candidates of proteins that interact with all of the beta1-like integrin cytoplasmic domains and may be instrumental for the formation of focal adhesion complexes, cell spreading and other adhesion-dependent cell shape changes. Various beta1-like integrins have different affinities to talin and filamin (45). Especially the beta1D cytoplasmic domain binds to talin and filamin with higher affinity that the other cytoplasmic domains. This might be important for the stability of adhesion contacts in muscle, compared to those in occasionally moving cells such as fibroblasts, endothelial cells and leukocytes.

Recently, we have studied the relationship between the ability of various beta1 cytoplasmic domain mutants to bind to talin in relationship to their ability to be organized in focal adhesions and to mediate cell spreading. We have found that point mutations at the NPXY motif disrupt both talin binding and focal adhesion organization. By using synthetic peptides we could verify the direct interaction of talin with the NPXY motif, as also published earlier (44). In our study, however, certain COOH-terminal deletion mutants were able to bind to talin, but were not able to form focal adhesions (Kääpä, Peter and Ylänne, submitted). This suggests that certain interactions requiring the sequences after the NPXY sequence are needed for proper integrin function. These results are summarized in figure 4. We have also constructed similar mutations in the cytoplasmic domain of beta3 (30) and beta2 (Ylänne and Peter, unpublished) and have found similar functional response to the COOH-terminal deletions. We have not been able to show interaction of filamin and alpha-actinin with this area. Thus our studies suggest that in several beta1-like cytoplasmic domains, the area after the NPXY motif would be required for formation of focal adhesions and for integrin-dependent cell spreading. This might be a conserved function for the beta1-like cytoplasmic domains, but no common binding partner mediating this function has yet been found.

Interestingly, two integrin-specific binding proteins have been shown to interact at, or close to, the functionally important area indicated in our studies. These are the beta1A-specific ICAP-1 (53) and the beta3-specific beta3-endonexin

(55; 56). The proteins are totally unrelated and very little is known about their role in signalling. It would be surprising, however, if a conserved function between various integrins would require specific interaction proteins with each of the integrin and these proteins would be unrelated.

In regard to conserved integrin-induced intracellular signals, I think that the role of cytoskeleton framework in the signalling has been underestimated. The importance of cytoskeleton in the signals have been mostly pointed out by the studies in which the microfilament disrupting drug cytochalasin D has been used (85-87). The studies show that most, if not all integrin-dependent tyrosine phosphorylation events are inhibited by the drug. Furthermore, cytochalsin inhibits the co-localization of several signalling molecules with integrin clusters (71; 88; 89).

## 9. PERSPECTIVES

It is possible that some components interacting with the of beta1-like integrin cytoplasmic domains are still missing. I would predict that one missing protein would interact with all beta1-like cytoplasmic tails and that the sequences at or before the NXXY motif on integrins would be needed for this interaction. This interaction might be necessary for integrin-mediated organization of focal adhesions and for cell spreading. The interaction site of this component overlaps with the region where the beta5 subunit has the 8-amino-acid insertion (figure 2), and most probably the decreased ability of beta5 to organize focal contacts might be explained by its reduced binding ability to this hypothetical protein.

The molecular events leading to the assembly of focal adhesions and to cell spreading are still not completely understood. Perhaps one of the problems is that the interactions are relatively weak and require clustering of both integrins and the interacting partners. Binding to multivalent extracellular ligands may be the way to cluster integrins and the cytoskeleton framework may help clustering the cytoplasmic binding proteins. This makes the analysis of interactions *in vitro* technically demanding, but the possibility to construct various recombinant marker molecules may help to solve these problems.

## 10 . ACKNOWLEDGEMENTS

Research in the author's laboratory has been supported by university of Helsinki and the academy of Finland.

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**Keywords:** Integrin, Cell Adhesion, Cytoskeleton, Signal Transduction

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