Cell adhesion in vascular biology. New insights into integrin-ligand interaction.

J C Loftus, R C Liddington

Perspective

Perspectives Series:
Cell Adhesion in Vascular Biology

New Insights into Integrin–Ligand Interaction

Joseph C. Loftus* and Robert C. Liddington‡
*The Mayo Clinic Arizona, Scottsdale, Arizona 85259; and ‡Department of Biochemistry, University of Leicester, Leicester LE1 7RH, United Kingdom

Introduction

Integrin mediated cell–cell and cell–matrix adhesion is at the root of a diverse range of physiological processes. An understanding of the molecular mechanisms of integrin–ligand interaction requires identification of the recognition sites within the macromolecular integrin ligands as well as the ligand contact points within the integrin receptor. A number of discrete recognition sequences within integrin ligands have been identified and characterized. Based on high resolution structures for several of these recognition sequences, an emerging theme is that these recognition sites consist of short peptide sequences presented on extended flexible loops between β strands (1–3). By contrast, an understanding of the sites within the integrin receptors that define ligand recognition and specificity has been slower to emerge. This is due in part to the complexity of these large heterodimeric molecules, the dynamic modulation of their ligand binding affinity, and their potential for allostery. It is generally accepted that integrins contain multiple ligand contact sites, as essential regions and specific residues have been identified in both the α and β subunits. The three major regions are the α subunit I-domain, the seven NH_2-terminal repeats of the α subunit, and a conserved region of the β subunits that appears to be a functional and structural homolog of the α subunit I-domain. Atomic resolution crystal structures exist only for the α subunit I-domain (4, 5), but recent hypothetical atomic models of the other two domains present an opportunity to build a quaternary model of integrin ligand binding and allostery in the light of the mutagenesis and biochemical data.

The α subunit I-domain. The first crystal structure of an α subunit I-domain demonstrated that it adopts the dinucleotide-binding fold, with a central parallel β sheet surrounded on both sides by α helices (5). This fold is very common among intracellular phosphoryl transfer enzymes, but has not been observed previously in an extracellular domain. In this class of fold, the functional surface of the molecules always lies at the COOH-terminal end of the β sheet. In the α subunit I-domain, a unique divalent cation coordination sphere is located there and has been designated the metal ion–dependent adhesion

site (MIDAS).[1] Interestingly, in the $α_M$ I-domain crystal structure with bound Mg^{2+}, a glutamate sidechain from a neighboring I-domain in the crystal lattice completed the octahedral coordination sphere of the metal, leading to the suggestion that the glutamate behaved as a ligand mimetic. This is consistent with the fact that all integrin ligands possess a critical aspartate residue (or glutamate) as a key feature of their integrin-binding motifs, and mutation of any of the metal-coordinating sidechains of the I-domain (6, 7) abolishes binding in a dominant negative fashion. The concept of a metal ion providing a bridge between ligand and receptor has been widely anticipated (8, 9), and the crystal data provide tantalizing though not direct proof of its existence.

Apart from the highly conserved oxygenated residues that directly coordinate the metal, the upper surface of the domains surrounding the MIDAS motif is highly variable. One might imagine that the metal-Glu/Asp bond contributes about half of the binding energy, with the rest of the energy and the specificity arising from further interactions (ionic/polar/hydrophobic) between complementary surfaces of the integrin and ligand. Two groups have set out to directly test this hypothesis by mutating residues on the MIDAS face. Huang and Springer (10) used mouse–human chimeras and site-specific mutagenesis to demonstrate that residues essential for the interaction of lymphocyte function–associated antigen–1 (LFA-1) with intercellular adhesion molecule–1 (ICAM-1) were located on the MIDAS face surrounding the site of metal coordination. Similarly, Rieu et al. (11) showed that residues essential for the binding of the hookworm pathogen, NIF, a protein that blocks the binding of natural ligands to $α_Mβ_2$, cluster around the MIDAS face of $α_M$.

The β subunit I–like domain. The most highly conserved region of the integrin β subunits is an $\sim$ 250 amino acid stretch located near the amino terminus. Strong support for this region of $β_3$ in ligand binding function is provided by the observations that arginine-glycine-aspartic acid–containing peptides cross-link to this region, mutations within this region abolish ligand binding, and several antibodies that inhibit ligand binding map to this region (12). Mutations in the corresponding region of other β subunits similarly block ligand binding, and function blocking as well as activating mAbs for $β_1$ map to a discrete region within this fragment (13).

The presence of an invariant $D \times S \times S$ sequence required for ligand binding within this region of the β subunits as well as within the α subunit I-domain led to the suggestion that these

---

Address correspondence to Joseph C. Loftus, Ph.D., The Mayo Clinic Scottsdale, 13400 East Shea Boulevard, Scottsdale, AZ 85259. Phone: 602-301-6274; FAX: 602-301-7017; or Robert C. Liddington, Ph.D.; Phone: 44-116-252-3364; FAX: 44-116-252-3473.

1. *Abbreviation used in this paper:* MIDAS, metal ion–dependent adhesion site.

two regions might be structurally and functionally related (14). Based on the similarity of hydropathy plots between the α subunit I-domain and this region of the β_3 subunit (5), a hypothetical atomic model was built of the β_3 subunit domain that predicts that this region is likely to share many of the structural features of the α subunit I-domain (15). Mutagenesis of candidate oxygenated residues in the β_3 subunit predicted to be involved in cation coordination and ligand binding fully support this model. Similar results obtained with the corresponding regions of β_2 (16) and β_1 (17) further support the hypothesis that this region of the β subunits adopts a similar but not identical fold to the I-domain and might engage ligands via a MIDAS-like motif. However, unlike the α subunit I-domain, direct evidence for metal binding to this region of the β subunits region has not been provided.

The α-chain sevenfold repeats. Recently, a hypothetical but very persuasive atomic model of the seven NH$_2$-terminal repeats of the α subunits has been built (18). This model was inspired by the crystal structure of the heterotrimeric G-proteins. The α subunit fold is predicted to be a β propeller, a cyclic structure made of seven modules that can fold as a unit but not independently. The ligand contact points are located on the upper surface of the propeller, and may include loops from more than one "blade," as well as the propeller axis. Interestingly, the much discussed "EF-hand" Ca^{2+}-binding motifs of the α subunits lie on the lower surface of the propeller far from the ligand contact sites. It is likely then that these play a structural rather than ligand-binding role, perhaps by connecting the propeller to the stalk regions. Alternatively, they may be involved in interactions with the β subunit.

Tertiary structure changes

There is abundant evidence that integrins change their conformations during ligand binding and activation (19–21). In principle, changes in affinity of a ligand-binding site could arise in two distinct ways: a movement of domains that "unmasks" the ligand binding site, or tertiary changes within the ligand-binding domain that alter the shape and charge properties of the interacting surface ("shape shifting"). Both mechanisms may operate within integrins; there is some evidence for shape shifting in the I-domains, while the propeller epitopes may simply be unmasked.

The dinucleotide fold adopted by the I-domain is well known in other systems for undergoing tertiary structure changes. Lee et al. (22) have shown that the α_M I-domain can adopt two different conformations depending on the solution conditions before crystallization. They showed that changes in metal stereochemistry are linked to changes in the shape and charge distribution of the MIDAS face and also to tertiary changes, which are propagated to the COOH-terminal helix and the opposite pole of the molecule. This led to the suggestion that the two structures represent active and inactive conformers of the I-domain. In this model, it is the presence or absence of a ligand mimetic that "decides" the conformation. Therefore, it is not contradicted by the lack of conformational changes observed in the crystal structures of the α_L I-domain, grown in the presence of different metals or in the absence of metal, which lack a ligand mimetic (23).

There is a wealth of evidence supporting overall conformational changes throughout the β subunit as the epitopes for many inhibitory or activating monoclonal antibodies map to the β subunit (24). These studies do not, however, distinguish

between unmasking and shape shifting. Within the β_3 subunit I-like domain, two monoclonal antibodies specific for the activated conformation of integrin $\alpha_{IIb}\beta_3$ that map at or near the ligand binding site provide support for conformational changes in this domain (25, 26). In addition, the dominant negative effect of mutations of the β subunit MIDAS-like residues provides strong evidence that shape shifting is required for attainment of the active quaternary conformation (see below).

Quaternary structure changes

We do not yet know how quaternary changes (i.e., the way in which the α and β chains pack against each other) are linked to structural changes in the ligand-binding domains, which alter their affinity for ligands. One possibility derives from the study of the heterotrimeric G-proteins. In that system, the βγ subunits form a propeller with ligand binding sites that are masked in the inactive state by the α subunit GTPase domain (similar to the I-domain fold). On hydrolysis of GTP, the GTPase domain changes its conformation and ligand binding properties, detaches from the βγ dimer, and unmasks ligand binding sites on the propeller (27). In an analogous model of integrin allostery, the β subunit I-domain structure would sit on top of the α subunit propeller in the low affinity quaternary state, where it sterically blocks the propeller ligand binding sites. In the high affinity quaternary state, the β subunit I-domain–like structure is released from its contacts with the propeller, leading to shape shifting in the β I-domain and unmasking of the propeller ligand binding sites (Fig. 1). In support of this model, we have obtained evidence that the synergy site of fibronectin, located in the ninth type III repeat (FIII9), binds to the third blade of the α subunit propeller of integrin $\alpha_5\beta_1$, while the RGD motif, located in FIII10, binds to the β subunit I-domain (Mould et al., personal communication). The structure of the fibronectin FIII7-FIII10 fragment has recently been solved and it places these two motifs 35 Å apart (28).
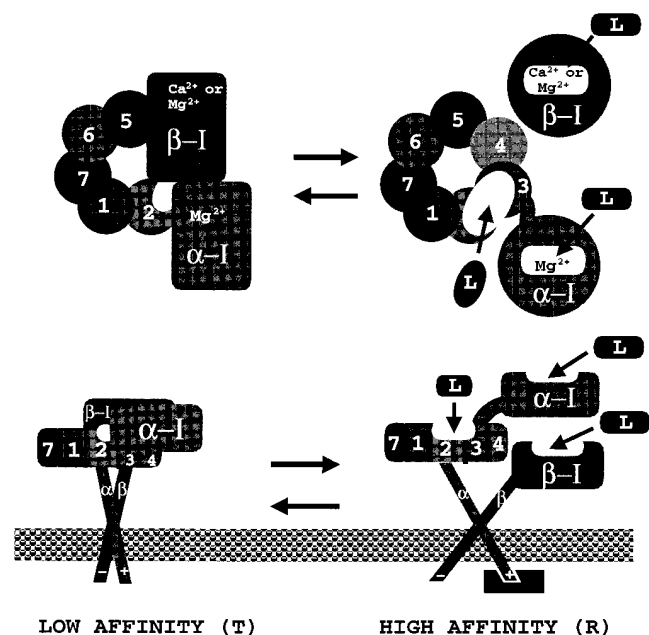

Figure 1. Top and side views of a hypothetical integrin with three ligand binding sites (*L*) in equilibrium between the low and high affinity quaternary states.

This places stringent constraints on the positions of the two integrin ligand binding sites and requires that, in the active state, the β subunit I-domain lies against the side of the α subunit propeller close to the third blade.

In those integrins that contain an α subunit I-domain, the propeller model places it also on top of the propeller, between the second and third blades, where it could obscure some of the propeller ligand binding sites. The conformational differences observed in the crystal structures of the $α_M$ I-domain are consistent with the idea that activation leads to a hinge motion of the I-domain that unmasks potential ligand binding sites on the propeller. This motion would also allow shape shifting within the I-domain that allows it to bind ligands with high affinity. If these two structures represent high and low affinity forms, then we must look for a surface on the I-domain that binds a "repressor" and locks the I-domain in the low affinity conformation in the resting integrin. Zhang and Plow (29) have made $α_M/α_L$ chimeras that lead to a constitutively active ligand binding function. The substitutions are on a surface of the I-domain distal to the MIDAS face, near the domain termini. The proposed α subunit β propeller blades may contact this region, but the "repressor" must also include the β chain, and it is possible that the β subunit I-like domain may also contact the α subunit I-domain. In this regard, it is noteworthy that mutations in the β subunit MIDAS motif effectively abolish ligand binding to $α_Mβ_2$ and $α_Lβ_2$ (16) despite the integrity of the ligand-binding epitopes of the α subunit I-domain. While such mutations might exert their effect by preventing the formation of an initial receptor/cation/ligand ternary complex (30), an alternative explanation is that these mutations lock the β subunit I-domain into the inactive conformer, which in turn locks the position of the neighboring α subunit I-domain; i.e., allosteric control of quaternary structure. This dominant negative effect is also seen in the binding of non–arginine-glycine-aspartic acid ligands to integrins that lack α subunit I-domains.

A "two-state" model

The emerging picture is that integrins have up to three, but typically two, distinct ligand binding sites that are intimately linked and allosterically controlled. The simplest allosteric model with which we could try to explain the ligand binding behavior of integrins is the two-state model formulated by Monod et al. (31). The two states refer to two distinct quaternary arrangements (T and R) of the αβ heterodimer. With one ligand binding site, this model generates four possible states of the integrin with different energies (unliganded T [Tu], liganded T [TL], unliganded R [Ru], and liganded R [RL]). With two ligand binding sites, there are six thermodynamic states.

The simplest way to visualize this model is in terms of energy "ladders" (Figs. 2 and 3). There is a T state ladder with narrowly spaced rungs (low affinity for ligand) and an R state ladder with broadly spaced rungs (high ligand affinity). Tu is in equilibrium with Ru and TL with RL. The quaternary conformation (T or R) of the integrin in the absence of ligand, the affinity for ligand, and the effect of ligand on conformation depends on the relative positions of the two ladders. For example, in Fig. 2 a, Tu is lower in energy than Ru and TL is lower than RL so that the integrin will remain in the T state (low affinity) whether the ligand is bound or not. In Fig. 2 b, the R ladder has lower energy, and ligand binding induces the
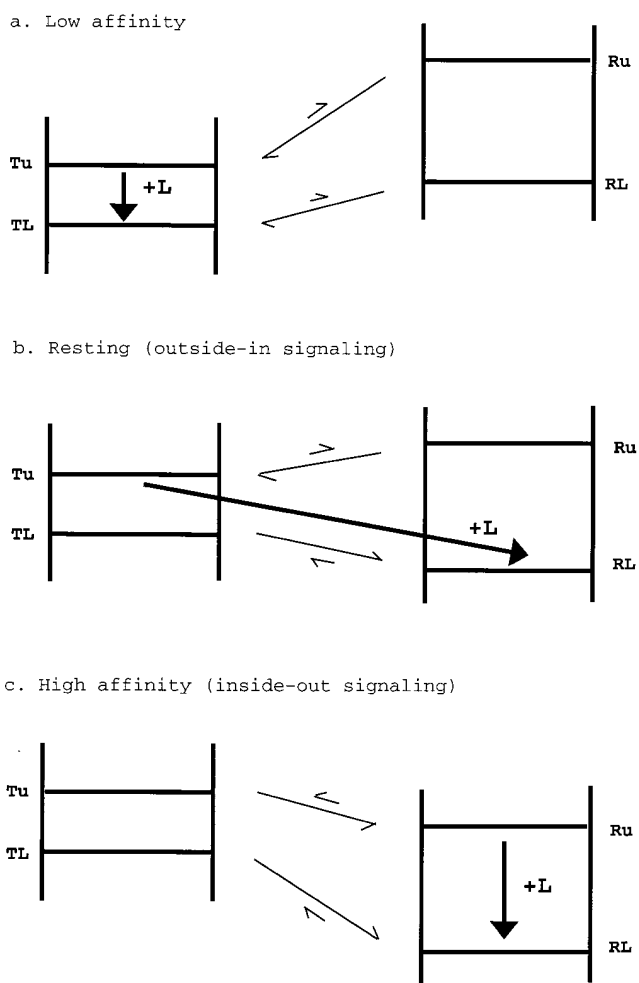

Figure 2. A two-state model of integrin allostery with one ligand binding site.

conformational switch; i.e., the condition for outside-in signaling. In Fig. 2 c, the R state has still lower energy. The integrin will remain in the high affinity state in the presence or absence of ligand, and ligand binding will induce only local structural effects; i.e., the condition in which the integrin has been activated by an intracellular signal (inside-out signaling).

In a fascinating series of experiments, Hughes et al. (32) did charge reversal mutations, indicating that a single salt bridge between the α and β cytoplasmic tails is required to stabilize the low affinity (T) conformation. Mutations affecting the putative salt bridge partners destabilize the T state, pushing the ladder upwards relative to the R state ladder, into a state resembling Fig. 2 c. A single salt bridge is probably worth $\sim$ 3 kcal/mol and corresponds to the difference in energy between Tu and Ru. If the ligand binding energies are in the nanomolar range ($\sim$ 8 kcal/mol) for the R state and micromolar ($\sim$ 4 kcal/mol) for the T state, this generates the condition in Fig. 2 b or 3 b in the resting integrin; i.e., poised for outside-in signaling. The similarities with hemoglobin are strong here. In that system, salt bridges also stabilize the low affinity T state, and mutations that break those salt bridges increase the affinity for oxygen by shifting the equilibrium towards the quaternary R state. In cells, integrin-associated proteins could act to stabilize the high affinity state by binding and sequestering
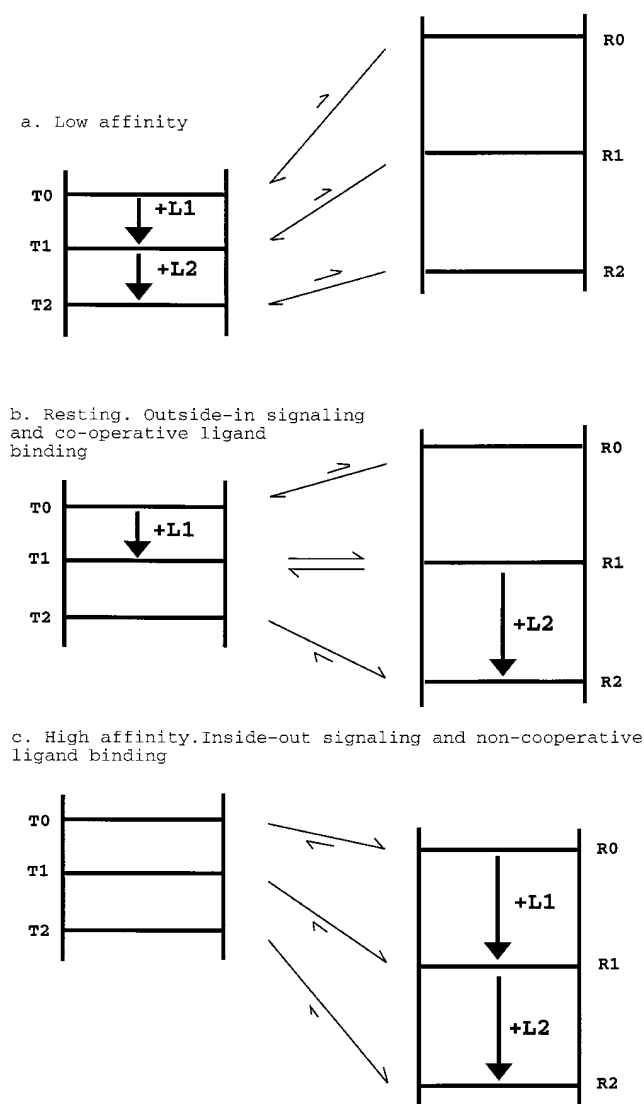
Figure 3. A two-state model of integrin allostery with two ligand binding sites.

---

one or both of the salt bridge partners leading to the condition in Fig. 2 *c* or 3 *c*. Since this system relies on the quaternary organization of the T state to reduce the intrinsic ligand binding affinity (the R state has the affinity expected of isolated domains), most mutations in the interface between the α and β subunits (intra- or extracellular) are likely to destabilize the T state and lead to a constitutively high affinity integrin locked in the R state (33, 34).

This simple model can provide a rationale for most natural and unnatural effectors. For example, the effect of activating antibodies can be explained in terms of stabilizing the R state (moving the right hand ladder down). While doing this, they may or may not block one or more of the ligand binding sites. An inhibitory antibody could exert its effect by stabilizing the T state, by directly obscuring a ligand binding site, or both.

In the case of two ligand binding sites, the situation becomes more complex. For example, there is the possibility of cooperative ligand binding (Fig. 3 *b*). Here, binding to the first site occurs with low affinity but induces a significant popula-

tion of the R state, and binding to the second site occurs with high affinity. One must also take into account synergistic binding whenever the ligand is bivalent and the two binding sites are the correct distance apart.

Conclusion

The breadth of integrin-mediated adhesive interactions and their critical biological implications have ensured an intensive effort directed at elucidating the molecular basis for integrin–ligand interaction. Recent studies have added significantly to the understanding of the interrelationships between ligand binding sites. A continuing analysis of integrin–ligand interactions together with emerging insights into the regulation of integrin affinity should provide new approaches to the control of cell adhesion.

Acknowledgments

References

1. Leahy, D., W. Hendrickson, I. Aukhil, and H. Erickson. 1992. Structure of a fibronectin type III domain from tenascin phased by MAD analysis of the selenomethionyl protein. *Science (Wash. DC).* 258:987–991.

2. Main, A., T. Harvey, M. Baron, J. Boyd, and I. Campbell. 1992. The three-dimensional structure of the tenth type III module of fibronectin: an insight into RGD-mediated interactions. *Cell.* 71:671–678.

3. Jones, E.Y., K. Harlos, M.J. Bottomly, R.C. Robinson, P.C. Driscoll, R.M. Edwards, J.M. Clements, T.J. Dudgeon, and D.I. Stuart. 1995. Crystal structure of an integrin binding fragment of the vascular cell adhesion molecule-1 at 1.8 Å resolution. *Nature (Lond.).* 373:539–544.

4. Qu, A., and D.J. Leahy. 1995. Crystal structure of the I domain from the CD11a/CD18 (LFA-1, $\alpha_L\beta_2$) integrin. *Proc. Natl. Acad. Sci. USA.* 92:10277–10281.

5. Lee, J., P. Rieu, M. Arnaout, and R. Liddington. 1995. Crystal structure of the A domain from the α subunit of integrin CR3 (CD11b/CD18). *Cell.* 80:631–638.

6. Michishita, M., V. Videm, and M. Arnaout. 1993. A novel divalent cation-binding site in the A domain of the β_2 integrin CR3 (Cd11b/CD18) is essential for ligand binding. *Cell.* 72:857–867.

7. Kamata, T., R. Wright, and Y. Takada. 1995. Critical threonine and aspartic acid residues within the I domains of β_2 integrins for interactions with the intercellular adhesion molecule 1 (ICAM-1) and C3bi. *J. Biol. Chem.* 270:12531–12535.

8. Corbi, A.L., L.J. Miller, K. O'Connor, R.S. Larson, and T.A. Springer. 1987. cDNA cloning and complete primary structure of the α subunit of a leukocyte adhesion protein. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:4023–4028.

9. Loftus, J.C., T.E. O'Toole, E.F. Plow, A. Glass, A. Frelinger III, and M.H. Ginsberg. 1990. A β_3 integrin mutation abolishes ligand binding and alters divalent cation-dependent conformation. *Science (Wash. DC).* 249:915–918.

10. Huang, C., and T.A. Springer. 1995. A binding interface on the I-domain of lymphocyte function associated antigen-1 (LFA-1) required for specific interaction with intercellular adhesion molecule-1 (ICAM-1). *J. Biol. Chem.* 270:19008–19016.

11. Rieu, P., T. Sugimori, D.L. Griffith, and M.A. Arnaout. 1996. Solvent-accessible residues on the metal ion-dependent adhesion site face of integrin CR3 mediate its binding to the neutrophil inhibitory factor. *J. Biol. Chem.* 271:15858–15861.

12. Loftus, J.C., J.W. Smith, and M.H. Ginsberg. 1994. Integrin-mediated cell adhesion: the extracellular face. *J. Biol. Chem.* 269:25235–25238.

13. Takada, Y., and W. Puzon. 1993. Identification of a regulatory region of the integrin β_1 subunit using activating and inhibitory antibodies. *J. Biol. Chem.* 268:17597–17601.

14. Bajt, M.L., and J.C. Loftus. 1994. Mutation of a ligand binding domain of β_3 integrin. Integral role of oxygenated residues in $\alpha_{IIb}\beta_3$ (GPIIb-IIIa) receptor function. *J. Biol. Chem.* 269:20913–20919.

15. Tozer, E.C., R.C. Liddington, M. Sutcliffe, A.H. Smeeton, and J.C. Loftus. 1996. Ligand binding to integrin $\alpha_{IIb}\beta_3$ is dependent on a MIDAS-like domain in the β_3 subunit. *J. Biol. Chem.* 271:21978–21984.

16. Goodman, T.G., and M.L. Bajt. 1996. Identifying the putative metal ion dependent adhesion site in the β_2 (CD18) subunit required for $\alpha_L\beta_2$ and $\alpha_M\beta_2$ ligand interactions. *J. Biol. Chem.* 271:23729–23736.

17. Puzon-McLaughlin, W., and Y. Takada. 1996. Critical residues for

ligand binding in an I domain-like structure of the integrin β_1 subunit. *J. Biol. Chem.* 271:20438–20443.

18. Springer, T.A. 1997. Folding of the N-terminal, ligand binding region of integrin α subunits into a β-propeller domain. *Proc. Natl. Acad. Sci. USA.* 94: 65–72.

19. Frelinger, A., III, X.P. Du, E.F. Plow, and M.H. Ginsberg. 1991. Monoclonal antibodies to ligand-occupied conformers of integrin $\alpha_{IIb}\beta_3$ (glycoprotein IIb-IIIa) alter receptor affinity, specificity, and function. *J. Biol. Chem.* 266: 17106–17111.

20. Sims, P.J., M.H. Ginsberg, E.F. Plow, and S.J. Shattil. 1991. Effect of platelet activation on the conformation of the plasma membrane glycoprotein IIb-IIIa complex. *J. Biol. Chem.* 266:7345–7352.

21. Kouns, W.C., P. Hadvary, P. Haering, and B. Steiner. 1992. Conformational modulation of purified glycoprotein GPIIb-IIIa allows proteolytic generation of active fragments from either active or inactive GPIIb-IIIa. *J. Biol. Chem.* 267:18844–18851.

22. Lee, J.-O., L.A. Bankston, M.A. Arnaout, and R.C. Liddington. 1995. Two conformations of the integrin A-domain (I-domain): a pathway for activation? *Structure (Lond.).* 3:1333–1340.

23. Qu, A., and D.J. Leahy. 1996. The role of the divalent cation in the structure of the I domain from the CD11a/CD18 integrin. *Structure (Lond.).* 4: 931–942.

24. Faull, R.J., and M.H. Ginsberg. 1995. Dynamic regulation of integrins. *Stem Cells (Basel).* 13:38–46.

25. Andrieux, A., M.J., Rabiet, A. Chapel, E. Concord, and G. Marguerie. 1991. A highly conserved sequence of the Arg-Gly-Asp binding domain of the integrin β_3 subunit is sensitive to stimulation. *J. Biol. Chem.* 266:14202–14207.

26. Shattil, S.J., J.A. Hoxie, M. Cunningham, and L.F. Brass. 1985. Changes in the platelet membrane glycoprotein IIb-IIIa complex during platelet activation. *J. Biol. Chem.* 260:11107–1114.

27. Lambright, D., J. Sondek, A. Bohm, N. Skiba, H. Hamm, and P. Sigler. 1996. The 2.0 Å crystal structure of a heterotrimeric G-protein. *Nature (Lond.).* 379:311–319.

28. Leahy, D.J., I. Aukhil, and H.P. Erickson. 1996. 2.0 Å crystal structure of a four-domain segment of human fibronectin encompassing the RGD loop and the synergy region. *Cell.* 84:155–164.

29. Zhang, L., and E.F. Plow. 1996. A discrete site modulates activation of I domains. *J. Biol. Chem.* 271:29953–29957.

30. D'Souza, S.E., T.A. Haas, R.S. Piotrovicz, D.E. McGrath, H.R. Soule, C. Cierniewski, E.F. Plow, and J.W. Smith. 1994. Ligand and cation binding are dual functions of a discrete segment of the integrin β_3 subunit: cation displacement is involved in ligand binding. *Cell.* 70:659–667.

31. Monod, J., J. Wyman, and J.-P. Changeux. 1963. Allosteric sytems and cellular control systems. *J. Mol. Biol.* 6:306–329.

32. Hughes, P., F. Diaz-Gonzalez, L. Leong, C. Wu, J. McDonald, S.J. Shattil, and M.H. Ginsberg. 1996. Breaking the integrin hinge. *J. Biol. Chem.* 271: 6571–6574.

33. Hughes, P.E., T.E. O'Toole, J. Ylanne, S.J. Shattil, and M.H. Ginsberg. 1995. The conserved membrane-proximal region of an integrin cytoplasmic domain specifies ligand binding affinity. *J. Biol. Chem.* 270:12411–12417.

34. Peter, K., and T.E. O'Toole. 1995. Modulation of cell adhesion by changes in $\alpha_L\beta_2$ (LFA-1, CD11a/CD18) cytoplasmic domain/cytoskeletal interactions. *J. Exp. Med.* 181:315–326.