Integrin ligands at a glance

Jonathan D. Humphries*, Adam Byron* and Martin J. Humphries‡

Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK

*These authors contributed equally to this article
‡Author for correspondence (e-mail: martin.humphries@manchester.ac.uk)

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Integrins are one of the major families of cell adhesion receptors (Humphries, 2000; Hynes, 2002). All integrins are non-covalently linked, heterodimeric molecules containing an α and a β subunit. Both subunits are type I transmembrane proteins, containing large extracellular domains and mostly short cytoplasmic domains (Springer and Wang, 2004; Arnaout et al., 2005). Mammalian genomes contain 18 α subunit and 8 β subunit genes, and to date 24 different α-β combinations have been identified at the protein level. Although some subunits appear only in a single heterodimer, 12 integrins contain the β1 subunit, and five contain αV.

Integrin function has been determined through a combination of cell biological and genetic analyses. On the cytoplasmic face of the plasma membrane, integrin occupancy coordinates the assembly of cytoskeletal polymers and signalling complexes; on the extracellular face, integrins engage either extracellular matrix macromolecules or counter-receptors on adjacent cell surfaces. These bidirectional linkages impose spatial restrictions on signalling and extracellular matrix assembly, and thereby integrate cells with their microenvironment. In turn, membrane-proximal interactions initiate more distal functions such as tissue patterning (extracellularly) and cell fate determination (intracellularly). Genetic analyses of engineered or natural mutations have confirmed key roles for integrins in tissue integrity, cell trafficking, and differentiation (Bouvard et al., 2001; Bokel and Brown, 2002).

Aims of this article

A characteristic feature of most integrin receptors is their ability to bind a wide variety of ligands. Moreover, many extracellular matrix and cell surface adhesion proteins bind to multiple integrin receptors (Humphries, 1990; Plow et al., 2000; van der Flier and Sonnenberg, 2001). In recent years, structure-function analyses of both

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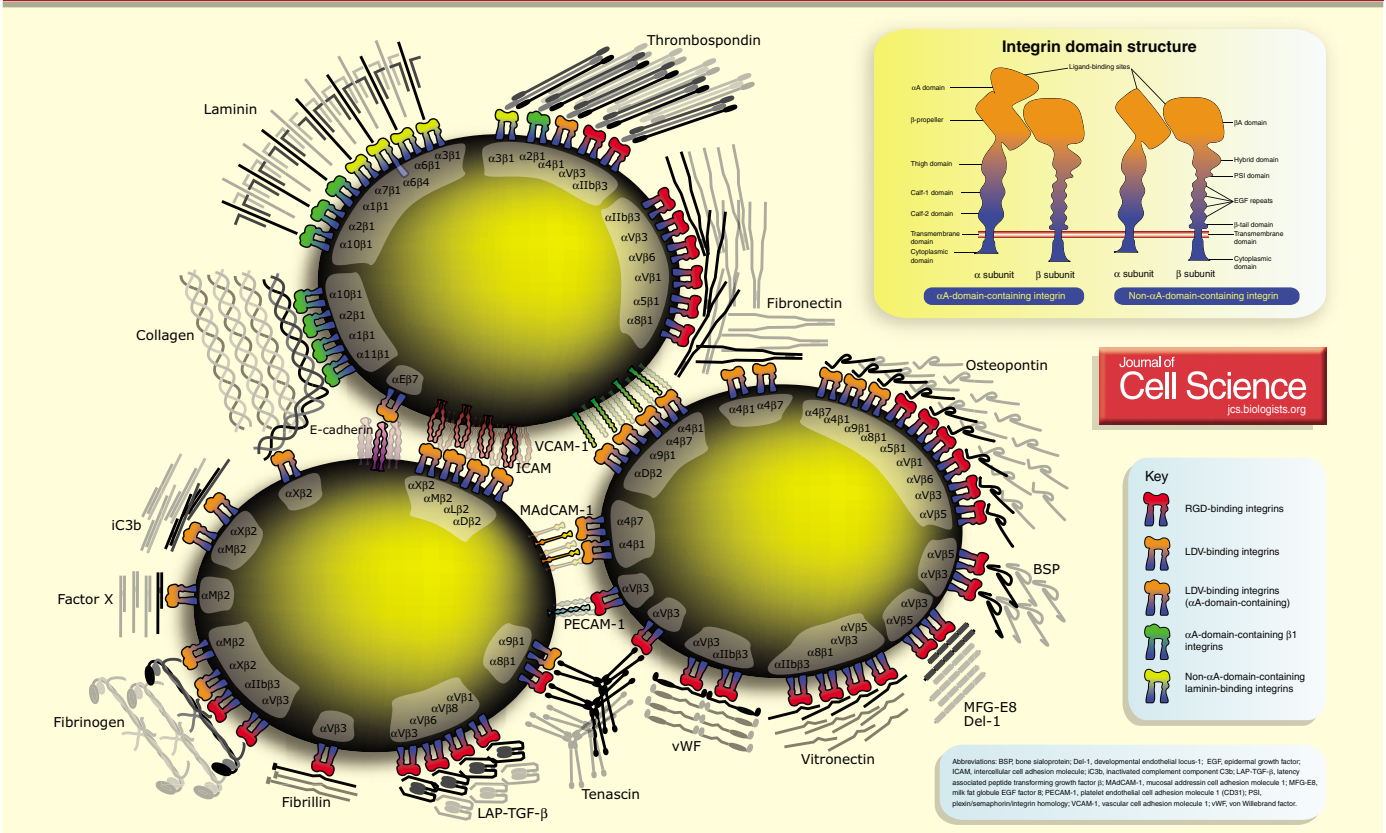

(See poster insert)

integrins and their ligands have revealed a similar mode of molecular interaction that explains this promiscuity. Nonetheless, the integrin literature is replete with studies describing different integrin-ligand pairs, and the major aim of this article is to provide a clarification of this picture.

The poster shows the major integrin-ligand combinations, using hypothetical cell surfaces. We have not attempted a comprehensive cataloguing, but instead we have consulted with a number of colleagues and reached a consensus view on the best-validated integrin ligands. There are many other ligands for different integrins, the inclusion of which would overly complicate the poster. By citing the best studied receptor-ligand combinations, we are aware that some reports and low-affinity interactions (which are nonetheless functionally relevant) may be discriminated against, and for this we apologise. Some of the interactions that are supported by convincing data are nonetheless included below.

Integrin-ligand partners

Historically, most integrin-ligand pairs have been identified either by affinity chromatography or through the ability of subunit-specific monoclonal antibodies to block adhesion of cells to specific ligands. In some cases, direct protein-protein binding assays have been used to support biochemical or cell biological data. Despite their wide variety, it is possible to cluster integrin-ligand combinations into four main classes, reflecting the structural basis of the molecular interaction. These classes do not necessarily reflect evolutionary relationships.

RGD-binding integrins

All five αV integrins, two β1 integrins (α5, α8) and αIIbβ3 share the ability to recognise ligands containing an RGD tripeptide active site. Crystal structures of αVβ3 and αIIbβ3 complexed with RGD ligands have revealed an identical atomic basis for this interaction (Xiong et al., 2002; Xiao et al., 2004). RGD binds at an interface between the α and β subunits, the R residue fitting into a cleft in a β-propeller module in the α subunit, and the D coordinating a cation bound in a von Willebrand factor A-domain in the β subunit. The RGD-binding integrins are among the most promiscuous in the family, with β3 integrins in particular binding to a large number of extracellular matrix and soluble vascular ligands. Although many ligands are shared by this subset of integrins, the rank order of ligand affinity varies, presumably reflecting the preciseness of the fit of the ligand RGD conformation with the specific α-β active site pockets.

LDV-binding integrins

α4β1, α4β7, α9β1, the four members of the β2 subfamily and αEβ7 recognise related sequences in their ligands. α4β1, α4β7 and α9β1 bind to an acidic motif, termed 'LDV', that is functionally related to RGD. Fibronectin contains the prototype LDV ligand in its type III connecting segment region, but other ligands (such as VCAM-1 and MAdCAM-1) employ related sequences. Although definitive structural information is lacking, it is highly likely that LDV peptides bind similarly to RGD at the junction between the α and β subunits. Osteopontin also interacts with α4β1, α4β7 and α9β1, but this apparently involves a different peptide motif, SVVYGLR, and the location of the ligand-binding site has not been identified.

The β2 family employ a different mode of ligand binding, the major interaction taking place through an inserted A-domain in the α subunit (see Shimaoka et al., 2003 for the structure of a complex between the αL A-domain and ICAM-1). However, despite this fundamental mechanistic difference, the characterised sites within ligands that bind β2 integrins are structurally similar to the LDV motif. The major difference is that β1/β7 ligands employ an aspartate residue for cation coordination whereas β2 integrins use glutamate. Collectively, therefore, the LDV motif can be described by the consensus sequence L/I-D/E-V/S/T-P/S.

A-domain β1 integrins

Four α subunits containing an αA-domain (α1, α2, α10 and α11) combine with β1 and form a distinct laminin/collagen-binding subfamily. Few other validated ligands have been identified for these integrins. A crystal structure of a complex between the α2 A-domain and a triple-helical collagenous peptide has revealed the structural basis of the interaction, a critical glutamate within a collagenous GFOGER motif providing the key cation-coordinating residue (Emsley et al., 2000). Currently, the mechanism of laminin binding is unknown.

Non-αA-domain-containing laminin-binding integrins

Three β1 integrins (α3, α6 and α7), plus α6β4, are highly selective laminin receptors. Analysis of laminin fragments indicates that these receptors and the A-domain-containing β1 integrins bind to different regions of the ligands. In neither case has the active site been narrowed down to a particular sequence or residue.

Additional integrin-ligand interactions

As discussed above, additional integrin ligands exist that, for the sake of clarity, we do not include in the poster, even though credible evidence exists for them. These ligands, along with their respective integrin partners, are therefore listed here: ADAM family members interact with α4β1, α5β1, α6β1, α9β1, αVβ3 and αVβ6; COMP interacts with α5β1 and αvβ3; connective tissue growth factor interacts with αVβ3 and αIIbβ3; Cyr61 interacts with α6β1, αIIbβ3, αVβ3 and αDβ2; E-cadherin interacts with α2β1; ESM-1 interacts with αLβ2; fibrillin interacts with α5β1; fibrinogen interacts with αDβ2; fibronectin interacts with αDβ2; ICAM-4 interacts with α4β1, αLβ2, αMβ2, αXβ2, αVβ3 and αIIbβ3; LAP-TGFβ interacts with α8β1 and αVβ5; MMP-2 interacts with αVβ3; nephronectin interacts with α8β1; L1 interacts with α5β1, αVβ1, αVβ3 and αIIbβ3; plasminogen interacts with αDβ2; POEM interacts with α8β1; tenascin interacts with α2β1; thrombospondin interacts with α5β1 and α6β1; VEGF-C and VEGF-D interact with α9β1; and vitronectin interacts with αDβ2. Note also that both αMβ2 and αXβ2 interact with heparin and negative charges in denatured proteins.

Lessons from evolution

The model invertebrates *Drosophila melanogaster* and *Caenorhabditis elegans* have a much smaller complement of integrins than vertebrates (Hynes and Zhao, 2000). *Drosophila* has two β subunits (βPS and β*ν*) and five α subunits. β*ν* has no known α subunit partner, but βPS combines with subunits that cluster with the laminin-binding and RGD-binding integrins. The remaining α chains form a *Drosophila*-specific clade. A similar complement of integrins is found in *Caenorhabditis elegans*, which suggests that the earliest metazoans possessed two primordial integrins: one laminin-specific and one RGD-ligand-specific.

The genome of the early chordate *Ciona intestinalis* encodes eleven α and five β chain genes (Ewan et al., 2005). Two *Ciona* α chains cluster with laminin-binding subunits and a third clusters with RGD-binding subunits. Surprisingly, eight α chains contain an αA-domain that is related to but, distinct from, the vertebrate αA-domains. Since these subunits are expressed predominantly in blood cells, they may play a role in innate immunity. It therefore seems that collagen-binding capabilities appeared in the chordate lineage after the divergence of ascidians. Of the five *Ciona* β chains, one clusters with β1, one clusters with β4, and three form an ascidian-specific clade.

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