- What are integrins?

  Integrins are receptors that are heterodimeric, cell surface glycoproteins. They are composed of 2 subunits, the alpha and beta subunits forming a dimer.

Integrin Family slide 1

- 24 integrins have been identified so far and this diversity in integrins is a result of the different pairings of 18 α- and 8 β-subunits. An additional point - each of the subunits have multiple additional domains.
- 9 of the 18 mammalian α subunits contain an additional vWFA domain. This is also known as αA or the I domain. 9 of the aforementioned integrins belong to the αA-containing subclass.
- The N-terminal of each of these two subunits forms a ligand binding globular head, connected to the membrane by a long (approx. 170 angstrom) stalk.
- Ligand binding to integrin requires divalent cations and involves solvent exposed aspartate or a glutamate in the αA-containing subclass.
- Integrins recognise a large number of physiologic ligands - including soluble and surface bound proteins.
- Integrin binding results in force generation at focal contacts which is the site where integrins and ligands bind which affects contractile apparatus of the cell and influences assembly and remodeling of extracellular matrix.

Integrin family slide 2

- There is diversity in integrin binding as well. High affinity binding to ligands needs a conformational switch. The switch to high affinity state is rapid (< 1 second) and reversible in less than a minute.

  Here is a brief overview of how integrin binding and signal transmission occurs -

- Inside out activation: Intracellular signals are generated in response to extracellular chemical/mechanical stresses. The signals are transported through short integrin cytoplasmic and transmembrane segments to extracellular ligand binding pockets that cause certain changes. High affinity binding is a result of reduction in dissociation rate.
- Outside in signalling: Once the integrin is bound to soluble/immobilized ligands, it forms micro or macro clusters and transmits signals inwards to reorganize the

cytoskeleton.

- Many integrins exist in 2 activation states (active and inactive) but some showcase intermediate affinity states.
- The dissociation rates of high affinity integrins are responsible for firm adhesion.
- The dissociation rates of low and intermediate affinity states allow some integrins to mediate cell rolling.
- Thus depending on their affinity state, integrins may mediate rolling or firm adhesion.

Integrin Functions -

- Cell adhesion - we just spoke about this. It is a result of high affinity binding
- Cell ECM interaction - signals are received from the ECM via ligand binding
- Bidirectional signalling - you have outside in and inside out signalling pathways within integrins
- Integration of external mechano chemical - cues
- The ability to rapidly and reversibly change from high to low affinity states of ligand binding

α subunit intro slide -

We spoke about the 9 α subunits that contain the vWFA domain which we called the αA domain.

-In αA containing integrins, the αA domain functions as the ligand binding domain

-To get a better idea of the structure of the αA domain, it is very similar to a GTPase, with the catalytic center replaced with a ligand-binding ‘MIDAS’ site. MIDAS stands for metal ion dependent adhesion site which as the name suggests requires a metal ion for ligand binding.

The Closed Conformation slide -

-the αA subunit exists in two conformations: closed and open.

In the closed conformation:

In the closed state, the architecture of these loops creates a coordination sphere that has a low affinity for physiologic ligands.

- Loop 1 ($\beta A-\alpha 1$ loop): Contains the invariant D140xSxS motif which coordinates the MIDAS metal ion (typically $Mn2+$).
- Loop 2 ($\alpha 3-\alpha 4$ loop): Features residue T209 (threonine residue), which in this state only hydrogen bonds to a metal-bound water molecule rather than coordinating the metal directly.
- Loop 3 ($\beta D-\alpha 5$ loop): Contains residue D242, which directly coordinates the metal ion.
- Resulting Configuration: This creates the "Thr-water-metal-Asp" closed MIDAS configuration, which is stabilized by the $F-\alpha 7$ loop remaining in a fixed position.

The open conformation slide -

- Loop 1: Moves inward by approximately 2 Å, shifting the position of the MIDAS motif. This configurational change creates an open site for glutamate from the ligand to bind with the MIDAS.
- Loop 2: The residue T209 moves to coordinate the metal ion directly ($Mg2+$or $Mn2+$).
- Loop 3: The residue D242 shifts its position to hydrogen bond to a metal-bound water molecule.
- Resulting Configuration: These movements result in the "Thr-metal-water-Asp" open configuration.

Structure of the ectodomain slide -

The integrin ectodomain is composed of 12 distinct domains: four within the αV-subunit and eight within the β3-subunit.
The N-terminal portions of these subunits combine to form a globular ligand-binding "head". The head is formed by the (β-propeller domain of the αV-subunit) and the (βA domain of the β3 subunit).
The remaining domains form two "legs".
An unexpected and significant structural feature is that these legs are bent at the "knees", folding back against the head.

Formation of the heterodimer slide -
The formation of an integrin heterodimer involves the assembly of one α-subunit and one β-subunit, which are held together at not just the head but through several interfaces spanning the entire molecule.

The Extracellular Head Interface
The primary and most significant intersubunit contact occurs within the globular "head" of the integrin, where the β-propeller domain of the α-subunit meets the βA domain of the β-subunit.
A specific arginine or lysine residue that protrudes from a helix of the βA domain directly into the center of the α-propeller channel. This residue is "caged" into place by two rings of primarily aromatic amino acids.

Transmembrane and Cytoplasmic Interaction

In addition to the head region, the subunits associate near and within the plasma membrane, particularly when the integrin is in its low-affinity (inactive) state:

- Transmembrane (TM) Segments: The α and β TM helices associate within the lipid bilayer.
- Cytoplasmic Tails: The short intracellular tails are held together by a combination of hydrophobic contacts and a specific electrostatic salt bridge.

These interactions between the two subunits is dynamic which is necessary because separation of transmembrane and cytoplasmic tails is required during high affinity binding.

The 3 cation sites of βA slide -

Now that we have seen how the heterodimer is formed, let's transition to ligand binding. Ligand binding involves 3 cation sites on the βA domain -

Difference between MIDAS, ADMIDAS and LIMBS -
1. MIDAS (Metal Ion-Dependent Adhesion Site)
   - Location: Situated on both αA and βA domains.
   - Primary Function: It is the primary ligand-binding site. It directly coordinates with a solvent-exposed acidic residue (aspartate or glutamate) from the ligand.
   - Mechanism: MIDAS undergoes a conformational switch between closed (low-affinity) and open (high-affinity) states.
   - Preferred Cations: Typically occupied by Mg2+or Mn2+; it has a very low affinity for the inhibitory Ca2+

2. ADMIDAS (Adjacent to MIDAS)
   - Location: Positioned adjacent to the MIDAS cation within the βA domain.
   - Primary Function: It acts as a dual-role regulatory site that can either stabilize the inactive state or promote the active state depending on the bound ion.

- Mechanism:
    - When bound to Ca2+, it generally stabilizes the unliganded (inactive) state and can act as an inhibitory site by increasing the rate of ligand dissociation.
    - When Mn2+ replaces Ca2+, or when ligand binding triggers a structural shift, ADMIDAS stabilizes the liganded state.
- Preferred Cations: Occupied by Ca2+in the inactive state and Mn2+ in the active/liganded state.

3. LIMBS (Ligand-Associated Metal-Binding Site)
    - Location: Found in the βA domain and only appears when the integrin is in its liganded state.
    - Primary Function: It serves as a positive regulator of high-affinity binding.
    - Mechanism: Its primary role is to stabilize the MIDAS metal once an activatory metal ion is bound.
    - Preferred Cations: Coordinates a third metal ion (typically Mn2+ or Mg2+) alongside MIDAS and ADMIDAS in the liganded structure.

Integrin ligand binding site slide -

We have seen the cation sites involved in ligand binding. Now we look at the site directly involved with ligand binding.

- Ligands interact with specific loops on the “top” of the β-propeller domain
- The Arg-Gly-Asp (RGD) motif is a common ligand sequence that takes part in the binding.
- The arginine (Arg) residue fits into a binding pocket within the β-propeller and promotes closer association of β-propeller and βA domains
- Asp residue provides the sixth coordination site for the metal ion at MIDAS

This interaction leads to activation of the MIDAS site and stabilization of the ligand-bound conformation

In integrins that possess an αA domain, this domain acts as an endogenous ligand for the βA domain. During activation, a specific glutamate residue at the end of the αA domain's α7 helix coordinates directly with the βA MIDAS metal, effectively locking the integrin into a high-affinity state.

LIgand binding and conformational change slide - (figure out later)
A review of the steps that took place -

1. RGD binds to a crevice between the β propeller and the βA domain.

Cytoplasmic tail slide -

The cytoplasmic tails of the α- and β-subunits are short but critical for regulating integrin affinity.

- In the inactive state, the α- and β-tails interact through a combination of hydrophobic contacts and a specific electrostatic salt bridge.
- Activation-Induced Dissociation: Intracellular activators like talin trigger a transition to the high-affinity state. Talin's PTB domain binds to the NPxY motif on the β-tail, which breaks the intersubunit salt bridge and dissociates the αβ interface. You need to break the salt bridge for activation.
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Transmembrane segments slide -

The TM segments play a vital role in transmitting signals across the plasma membrane:

- Length and Orientation: These segments are approximately 25–29 amino acids long. To accommodate this length within the membrane, they are likely tilted or coiled. EM images suggest that in the low-affinity state, these helices are oriented almost perpendicular to the long axis of the integrin legs.
- TM Interface and Activation: In the low-affinity state, the α and β TM helices associate, possibly in a coiled-coil arrangement or through GxxxG-like motifs. Activation involves a reorganization of this interface. Some models suggest the helices rotate or realign, while some studies suggest they may separate laterally by a small distance.

Ectodomain - TM connection slide -

The orientation and tilt of the integrin transmembrane (TM) helices are critical for ensuring the receptor is correctly positioned to interact with its environment.

Shift from Parallel to Tilted Orientation

Traditionally, structural models depicted integrin TM helices as being parallel to the long axis of the "legs" of the ectodomain. However, this configuration was problematic because it would result in the ligand-binding pocket facing the plasma membrane, rendering it inaccessible to extracellular ligands.

Current models, supported by three-dimensional reconstructions of EM images, show that the TM helices are not parallel but are actually oriented almost perpendicular or significantly tilted (approximately 35–40°) relative to the long axis of the legs. This specific orientation is vital because it ensures the ligand-binding site faces outward into the extracellular space, allowing the receptor to function as a mechanochemical sensor.

Longer the TM more is the tilt required to accommodate it in the membrane.

Functional Implications
This tilted orientation is a hallmark of the low-affinity (inactive) state. In this state, the long TM segments are likely embedded and tilted, stabilized by the salt bridge. Reorganizing this tilt—specifically shortening the TM segment by three to five amino acids or changing the tilt angle—is a central component of the inside-out activation process that switches the integrin to its high-affinity state.

Inside Out vs Outside In signalling slide -

Inside Out signalling -
Talin and kindlin binds to the integrin tail – transition from bent to extended state – the extended state enables high affinity ECM binding – promotes ECM binding and tissue remodelling

Outside In signalling -
Ligand binds – integrin clustering initiated – these influence signalling cascades – intracellular changes

The Deadbolt Model slide -

The deadbolt model suggests that bent integrins can bind ligands.

- Mechanism: It proposes that the CD loop of the βTD (β tail) domain acts as a "deadbolt," locking the βA domain in a low-affinity state by contacting its F−α7 region.

  Then how is the high affinity state achieved?
- Activation occurs when inside-out signals change the TM tilt angle, when the angle changes, the mechanical signal is transmitted to the βA domain. We saw earlier that the F-α7 and ADMIDAS binding maintains the low affinity state. Due to the mechanical signal, the F-α7 disengages from the ADMIDAS coordination which then causes structural changes in βA. The α1 helix (located on the βA) moves inwards as a result of which βA transitions to a high affinity state directly disengaging the deadbolt without requiring full leg extension. This model is considered more energetically favored for rapid, reversible signaling.

The Switchblade Model slide -

The integrin is initially in its inactive state in the similar way as in the deadbolt model. Just a point to note - the low affinity state is stabilized by a Ca2+ ion associated with the ADMIDAS.

Let’s look at the integrin domains in the intracellular region. In the inactive state, the α and β tails are associated with each other. Intracellular signals cause talin to bind the β tail. Talin binding causes disruption of the salt bridge and hydrophobic interactions which cause the separation or tilt of the cytoplasmic and TM domains and the signal is transmitted to the ectodomain. The hybrid domain swings out leading to a high affinity state which is stabilized by the binding of the Mg2+ or Mn2+ to MIDAS.

After ligand binding, a response is elicited.

Affinity vs Avidity slide -

Basic only - look at slide once