Adhesion receptors and mechanosensitive signaling

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Integrins

- Plasma membrane receptors

- Interconnects the ECM to the actin cytoskeleton to regulate force transduction

- Large extracellular domain that binds to ECM proteins

- Small intracellular domain that lacks intrinsic kinase activity but recruits signaling molecules

Several coordination sites for Mg2+ and Mn2+ (latter used to activate integrins in vitro)
Integrin activation

See Moser et al. Science Vol. 324, pp.895-899
Integrin heterodimerization determines ligand specificity

24 different combinations
Ligand specificity is determined by the combination of alpha and beta subunits

a5b1: FN
a1b1, a2b1: collagen
avb3: vitronectin
a3b1: FN, LM

Cell and Tissue Research Vol. 339 Issue 1
DOI: 10.1007/s00441-009-0834-6
Integrin signaling:

**Inside-out signaling:** the intracellular mechanism by which integrins are activated so they can bind ECM protein (ligand)

**Outside-in signaling:** how ECM transduces information into the cell
Integrin signaling complexes

Focal contacts
- talin
- initial adhesion
- force independent

Focal complexes
- talin, vinc
- Rac-dependent
- vinculin stabilizes FC and confers force-dependence

Focal adhesions
- talin, vinc, and many others;
- Rho-dependent slow to mature (~1 h)

IMAGE COURTESY OF RAFAEL GARCIA-MATA
The primary adhesion receptors are heterodimeric (α and β) integrins, represented by orange cylinders. Additional membrane-associated molecules enriched in these adhesions (red) include syndecan-4 (Syn4), layilin (Lay), the phosphatase leukocyte common antigen-related receptor (LAR), SHP-2 substrate-1 (SHPS-1) and the urokinase plasminogen activator receptor (uPAR). Proteins that interact with both integrin and actin, and which function as structural scaffolds of focal adhesions, include α-actinin (α-Act), talin (Tal), tensin (Ten) and filamin (Fil), shown as golden rods. Integrin-associated molecules in blue include: focal adhesion kinase (FAK), paxillin (Pax), integrin-linked kinase (ILK), down-regulated in rhabdomyosarcoma LIM-protein (DRAL), 14-3-3 and caveolin (Cav). Actin-associated proteins (green) include vasodilator-stimulated phosphoprotein (VASP), fimbrin (Fim), ezrin–radixin–moesin proteins (ERM), Abi kinase, nexitillin (Nex), parvin/actopaxin (Parv) and vinculin (Vin). Other proteins, many of which might serve as adaptor proteins, are coloured purple and include zyxin (Zyx), cysteine-rich protein (CRP), palladin (Pall), PINCH, paxillin kinase linker (PKL), PAK-interacting exchange factor (PIX), vinexin (Vnx), ponson (Pon), Grb-7, ASAP1, syntenin (Synt), and syndesmos (Synd). Among these are several enzymes, such as SH2-containing phosphatase-2 (SHP-2), SH2-containing inositol 5-phosphatase-2 (SHIP-2), p21-activated kinase (PAK), phosphatidylinositol 3-kinase (PI3K), Src-family kinases (Src FK), carboxy-terminal src kinase (Csk), the protease calpain II (Calp II) and protein kinase C (PKC). Enzymes are indicated by lighter shades.
Some of the signaling pathways located downstream of integrin activation.
A really important concept in signaling…. 

<table>
<thead>
<tr>
<th>MOTIF</th>
<th>BINDING TO:</th>
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<tbody>
<tr>
<td>SH2</td>
<td>phosphotyrosine sequences</td>
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<tr>
<td>PTB</td>
<td>phosphotyrosine sequences</td>
</tr>
<tr>
<td>SH3</td>
<td>proline-rich sequence</td>
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<tr>
<td>WW</td>
<td>proline-rich sequence</td>
</tr>
<tr>
<td>PH</td>
<td>phosphoinositol lipids</td>
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Clustering of integrins results in FAK transphosphorylation (autophosphorylation) at Y397

PhosphoY397 is a binding site for Src which then phosphorylates FAK at Several other tyrosines

These multiple phosphosites then dock other signaling molecules, some of Which get phosphorylated by FAK

There are several Ser/Thr phosphorylations In FAK but their role is not understood

taken from Parsons, JCS
Mechanosensing by FAK

cyclic stretch (to 115% at 1 Hz) for 10–120 min

Torsoni et al. AJP - Heart 289: H1488-H1496

Stiff: ~20,000 Pa
Soft: ~2,000 Pa

<table>
<thead>
<tr>
<th>Serum</th>
<th>stiff</th>
<th>soft</th>
<th>stiff</th>
<th>soft</th>
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<tr>
<td>-</td>
<td>3hrs</td>
<td>9hrs</td>
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</table>

- p130Cas<sup>Y410</sup>
- p130Cas
- pFAK<sup>Y397</sup>
- pFAK<sup>Y576</sup>
- pFAK<sup>Y861</sup>
- FAK
- pSrc<sup>Y418</sup>
- pSrc<sup>Y529</sup>
- Src
- pPaxillin<sup>Y118</sup>
- Paxillin
- GAPDH
Mechanosensing by p130Cas

In vitro 130Cas mechanosensor

Cas SD attached to biotin, in turn attached to flexible avidin-coated surface which was stretched to apply force
Mechanical regulation of transcription by YAP/Taz

JCB 193: 633-642 (2011)

Mechanical regulation of transcription by MRTF

Cadherins and adherens junctions (AJs)

Homophilic binding
Calcium dependent
Focal adhesions (FAs) vs. adherens junctions (AJs) regulate forces within AND BETWEEN cells

A. Focal adhesion
- Integrin
- Extracellular matrix
- F-actin

B. Adherens junction
- β-catenin
- p120-catenin
- Type I cadherin
- α-catenin
- F-actin

C. Actin-myosin network
- Myosin II
- F-actin
Crosstalk between FAs and AJs regulate force-dependent signaling
Rho family GTPases

- Rho-GTP
- Rho-GDP

- Rac-GTP
- Rac-GDP

- Cdc42-GTP
- Cdc42-GDP

Stress Fibers
Rho kinase

Lamellipodia
Pak

Filipodia
Pak
ACTIN CYTOSKELETON DYNAMICS: THE ROLE OF RHO-FAMILY G PROTEINS

Common structure of small G proteins allows design of constitutively active (CA) and dominant negative (DN) mutant proteins

CA-RhoA, CA-Rac, CA-Cdc42 expressed in fibroblasts induce specific architectural structures

FOCAL ADHESIONS & STRESS FIBERS

LAMELLIPODIA

FILOPODIA
LifeAct MEFs on a fibronectin-coated surface
Rho Effector Pathways

- Rho
  - mDia
  - profilin
  - PKN
  - PI4P 5-K
- Rho kinase
  - MLCPase
  - MLCK
  - MLC
  - MLC-P + actin
  - cofillin
  - cofillin-P (inactive)
  - LIMK
- citron kinase
- rhophilin
- rhotekin
- MLC
- Y27632
- Blebbistatin

actin polymerization
contractility
actin depolymerization
Pulling it all together......

**Inside-out:** Rho-dependent contractility can contribute to the clustering of integrins and maturation of FAs

**Outside-in:** Integrin signaling regulates Rho activity

**Cadherin-integrin balance:** The connections between cadherin/a-catenin/actin and integrin/FA/actin means that there can be crosstalk between cell-substratum and cell-cell signaling.

Rho-dependent contractility can play an important role in regulating this crosstalk by changing actomyosin-mediated intracellular tension.
END