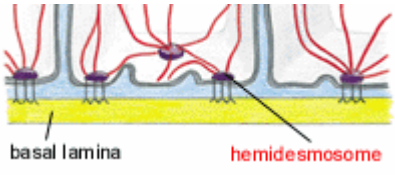
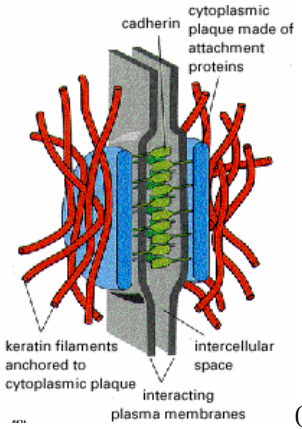
# Q1.

<table>
<tr><th></th><th>Hemidesmosomes</th><th>Desmosomes</th></tr>
<tr><td rowspan="2">Biochemistry</td><td colspan="2">Protein complex crossing the membrane</td></tr>
<tr><td>Transmembrane receptor is from intergin superfamily (specifically beta4 intergin).</td><td>Transmembrane receptor is from cadherin family (desmoglein, desmocollin, etc.).<br>Use $Ca^{2+}$-dependent mechanism to hold the adjacent membranes together.</td></tr>
<tr><td rowspan="2">Function</td><td colspan="2">These anchoring junctions function as connection sites for intermedia filaments.</td></tr>
<tr><td>Mediate cell-to-substrate adhesion;<br>Bind cellular intermediate filaments (plaque proteins on cyto-side are plectin, BP230, etc.) to extracellular matrix.</td><td>Mediate cell-to-cell connection;<br>Hold cell together by connecting bundles of actin filaments or intermediate filaments (plaque proteins on cyto-side are desmoplakins, plakoglobin, etc.).</td></tr>
<tr><td rowspan="2">Structure</td><td colspan="2">Button like points, riveted with intercellular filaments.<br>There are anchor proteins between transmembrane receptor proteins and intermediate filaments.</td></tr>
<tr><td>Hemidesmosome = “half desmosome”<br>  <br><br>(MBOC4, 1074)</td><td>  <br><br>(MBOC4, 1073)</td></tr>
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# Q2A.

**i).** Individual collagen polypeptide is synthesized on membrane-bound ribosome and injected into the lumen of endoplasmic reticulum by the amino-terminal signal peptide. Deletion of the “pre” sequence will block the generation of collagen: on one side, synthesized collagen polypeptide will be accumulated in the cytosol and then deliver to degradation; on the other side, the preexisting normal collagens lacking renew will be destroyed or degraded, which influences cell adhesion or cell motility, and causes the cell become fragile little by little.

**ii).** Propeptides at both the N- and C-terminal ends of the collagen polypeptides help to form the triple-stranded procollagen. Deletion of the “pro” sequence will make collagen polypeptides have problem in forming triple-helix. These polypeptides in the lumen of ER will be mis-formated or not-formated into triple-helix, which will be sent to degradation later. The normal existing collagen lacking renew will be degraded, the cell will become fragile little by little, and cell adhesion or motility will change.

**iii).** Cleavage of propeptides to generate functional collagen molecules is very important for further collagen fibril self-assembly. Mutations causing the “pro” peptides not be correctly cleaved may have two kinds of influences. One is the propeptides stay there and the collagen molecules can not be formed and no new collagen fibrils generated. The other is that the propeptides are cleaved incorrectly, maybe cleaved more than necessary, and the collagen molecules are formed but can not function correctly. These mutated fibrils influence the normal function of collagen, causing the cell be too fragile or too tough.

**iv).** Procollagen formation needs selected prolines and lysines to be correctly hydroxylated, which helps to stabilized the collagen. Mutation influencing the prolyl hydroxylase will cause the two kinds of effects. One is inappropriate hydroxylation of some un-necessary amino acid residues causing propeptides can not be cleaved. The other is that mutation can not hydroxylate prolines, and form unstable collagens, which can be degraded very easily and influence the rigidity of the cell.

# Q2B.

**i).** Proline, which has a ring structure, can stabilize the helical conformation of each collagen alpha chain. Glycine is the smallest amino acid, and allows the three helical alpha chains to pack tightly together to form the final collagen superhelix.

**ii).** Fibronectin type III (FnIII) repeats will be spliced in alternative ways, which is important for various functions. And actually after the splicing, only one repeat is required in adhesion recognition. There are many different receptors, which may be differentially expressed on different cell types, recognize different FnIII repeats and regulate cell adhesion.

There are many interactions in mediating adhesion, and a large complex should be generated during adhesion, which contains interactions of cell-to-cell and cell-to-ECM. FnIII is involved in these interactions, and different FnIII repeats have specific binding sites for other matrix macromolecules and for receptors on the surface of cells. So there usually only one FnIII repeat is predominant in adhesion of fibroblasts, and other FnIII repeats function as assistants.

**iii).** Laminin consist of various disulfide-bonded subunits. It contains an alpha chain linked to beta and gamma chains by disulfide bonds forming a triple-helical coiled-coil structure comprising the long arm of a distinctive cross-shaped molecule (rod-like domain); the short arms consist of the N-terminal portions of each subunit.

Highly ordered sequences in rod-like domain form disulfide bonds which stable the heterotrimer. Cystines are important for disulfide bonds formation, and there are also several hydrophobic amino acid residues contained in the rod-like domain to form hydrophobic interaction to help stable the heterotrimer. As reported in some papers (*JBC*, 266:12087), *p20* [RNIAEIIKDI], *PA22-2 (IKVAV)* [SRARKQAASIKVAVSADR] and *LRE* site [LRE] sequences, which are close to the C-terminus in the rod-like domain, are very important for receptor recognition and laminin stabilization.

The N-terminus of each subunit is different from each other, and causes the diversity of laminin, which is the basic of laminin adhesion function in various cell types.

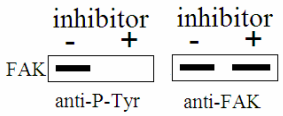
# Q3.

**i).** The Rho family protein couples extracellular signaling events to changes in cellular function. And a GTP-binding protein will be activated when binds to GTP by changing the conformation. Here, I would like to name the protein isolated as XYZ.

I would like to use GTP loading assay to measure activation of XYZ. Transfect cells (*XYZ*-) with XYZ expression construct (cells transfected with blank vector as control). Label cell cultures with [$^{32}$P]orthophosphate, and immunoprecipitate expressed XYZ using an anti-XYZ antibody. Use extensive wash to elute bound nucleotides, separate them on polyethyleneimine-cellulose TLC plates, and quantitate the autoradiograph results. The activation of XYZ is determined as the percentage of bound nucleotide corresponding to GTP, using the formula (Percent of Activation) = [$^{32}$P-GTP]/([$^{32}$P-GDP]+[$^{32}$P-GTP]). If we can't get *XYZ*- cells, we can use siRNA to knockdown the expression of XYZ for control.

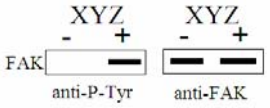
The activation of GTP-binding protein may increase in intracellular pH or cytoplasmic calcium, which can be measure by specific dye under fluorescent microscopy. As we don't know exactly how XYZ affects the in cellular function, using GTP loading assay to measure activation of XYZ is the best choice.

**ii).** Transmitting information from the extracellular compartment into the cell is called "outside-in signaling". The intracellular signaling cascades are activated when integrins bind to their extracellular ligands (*JBC*, 274:32486).




I will use specific inhibitor to XYZ to do the experiments to see whether inhibition of XYZ will block FAK (a well known kinase in the outside-in signaling via integrins) phosphorylation or not. The cells (*XYZ*-, expressing specific integrin) transfected with XYZ expression construct (cells transfected with blank vector, and cells transfected with XYZ but don't express specific integrin should be used as controls) are treated with the inhibitor. Collect cells; make lysate and SDS-PAGE. Do duplicate immunoblotting with anti-FAK antibody and anti-P-Tyr (anti-phosphotyrosine) antibody. If we can get the same results (not include the controls) as shown here, I would like to say that XYZ participates in outside-in signaling via integrins, because inhibition of XYZ blocks the FAK's tyrosine phosphorylation in the "integrin outside-in signaling". We could also attach cells to ECM (fibronectin *etc.*) coated substrate to stimulate integrin signaling, instead of expression specific integrin to activate signaling.

**iii).** The process of cell attachment and spreading also involves an "activation" of integrins themselves ("inside-out signaling"). This activation promotes cell adhesion and may be an important step in the morphological changes that cells undergo when spreading on a solid substrate.




The cells (*XYZ*-, DO NOT express specific integrin) transfected with XYZ expression construct (cells transfected with blank vector, and cells express specific integrin should be used as controls). Collect cells; make lysate and SDS-PAGE. Do duplicate immunoblotting with anti-FAK antibody and anti-P-Tyr antibody. If we can get the same results as shown here (not include positive control), I would like to say that XYZ participates in inside-out signaling via integrins, as activation of XYZ promotes the FAK's tyrosine phosphorylation just as integrins do in inside-out signaling. I think we may see some morphological differences between cells express XYZ or not. We could also use inhibitory antibodies or dominant negative to interfere integrins expression and signaling. If XYZ participates in integrin inside-out signaling, expression XYZ will overcome

interferences to integrins.

**iv).** I will use calcium switch and XYZ/cadherin co-localization analysis, as cadherin mediates calcium-dependent cell-cell adhesion. With normal $Ca^{2+}$ levels, EGFP-XYZ in its active GTP*-bound form can be detected (immunoprecipitation and autoradiograph). When cadherin-dependent adhesion is disturbed by $Ca^{2+}$ chelation, the level of active XYZ (GTP*-bound) will decrease. Also the amounts of active XYZ (GTP*-bound) will rapidly increase when cadherin mediated cell adhesion restores. We should also use confocal fluorescent microscopy to localize the subcellular location of EGFP-XYZ during calcium switch. If the recruitment of XYZ localized with accumulation of cadherin, we can say that XYZ is activated in response to cadherin-dependent adhesion.

**v).** <u>If XYZ positively regulates cadherin-dependent adhesion:</u> XYZ stabilizes (maybe through blocking beta-catenin's phosphorylation by inhibiting GSK3) beta-catenin (a cadherin associated protein locates at the adhesion site) to stable cell adhesion; beta-catenin interacting with TCF, translocates into nuclear, and functions as transcription factor, which binds to promoters and regulates expression of proteins involved in cell adhesion. XYZ may also participate in the anchorage of cadherin to the cytoskeleton and regulate cell adhesion. <u>If XYZ negatively regulates cadherin-dependent adhesion:</u> XYZ stimulates beta-catenin to be phosphorylated, ubiquitinated and degraded, and de-stabilizes cadherin-dependent adhesion.