Molecular Mechanism of Platelet Activation

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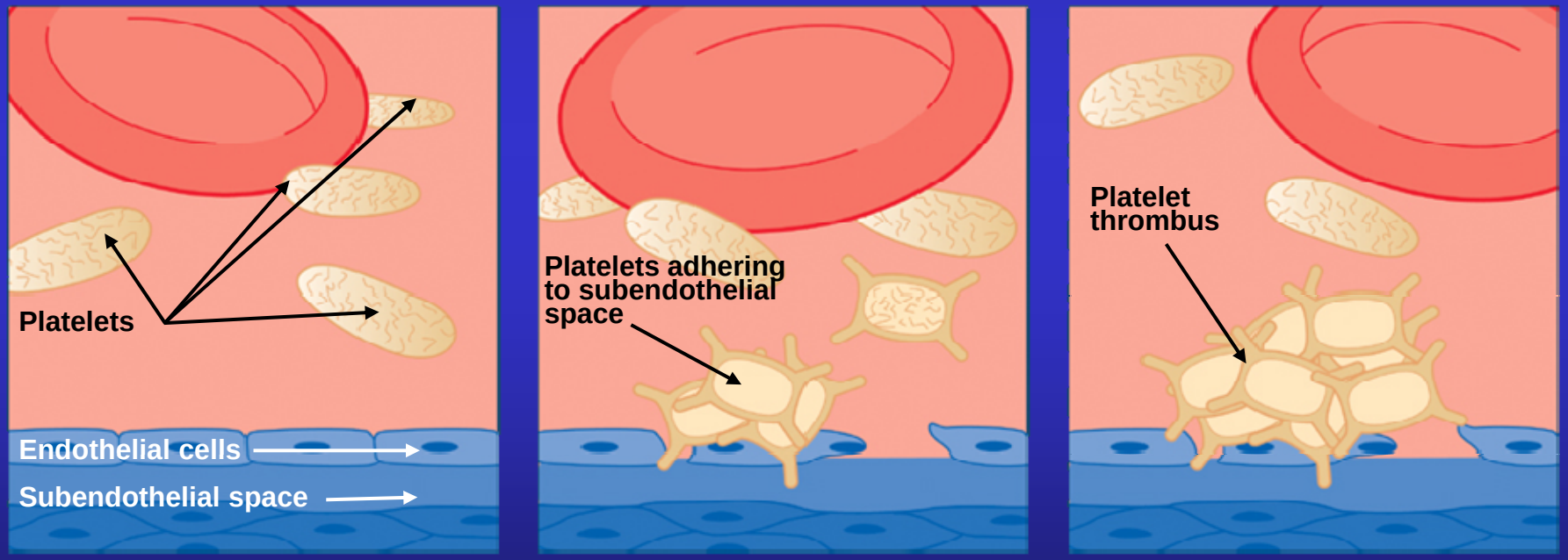
Platelet Adhesion, Activation and Aggregation

Normal platelets in flowing blood

Platelets adhering to damaged endothelium and undergoing activation

Aggregation of platelets into a thrombus



NO, PGI_2, Ectonucleotidase

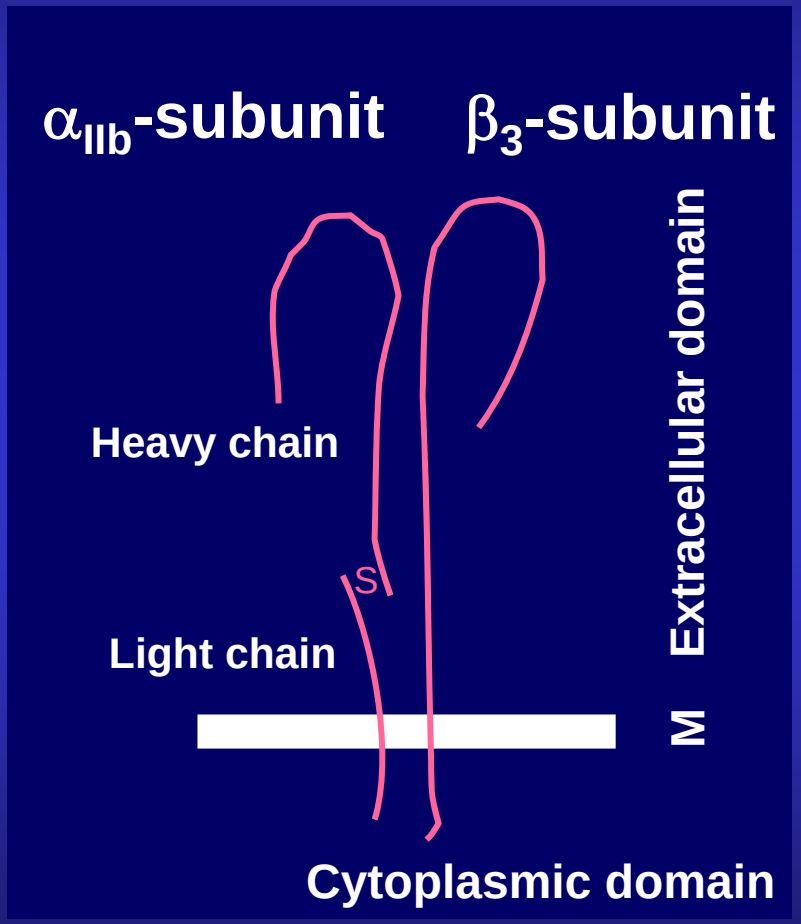
Adapted from: Ferguson JJ. *The Physiology of Normal Platelet Function*. In: Ferguson JJ, Chronos N, Harrington RA (Eds). *Antiplatelet Therapy in Clinical Practice*. London: Martin Dunitz; 2000: pp.15–35.

Platelet Activation

- Rapid changes in platelet morphology
  - From smooth disks into irregular spheroids
  - Extrusion of filopodia, which not only enhance adhesion but also are rich in GP IIb/IIIa receptors
- Granule secretion (ADP), and generation of thromboxane A_2
- Involvement of the cell surface in coagulation reactions; thrombin generation
- Platelet aggregation

Integrin

- α and β subunits
- Active and inactive state
- L-arginyl-L-glycyl-L-aspartate (RGD)
- “Inside out” signaling
- “Outside in” signaling



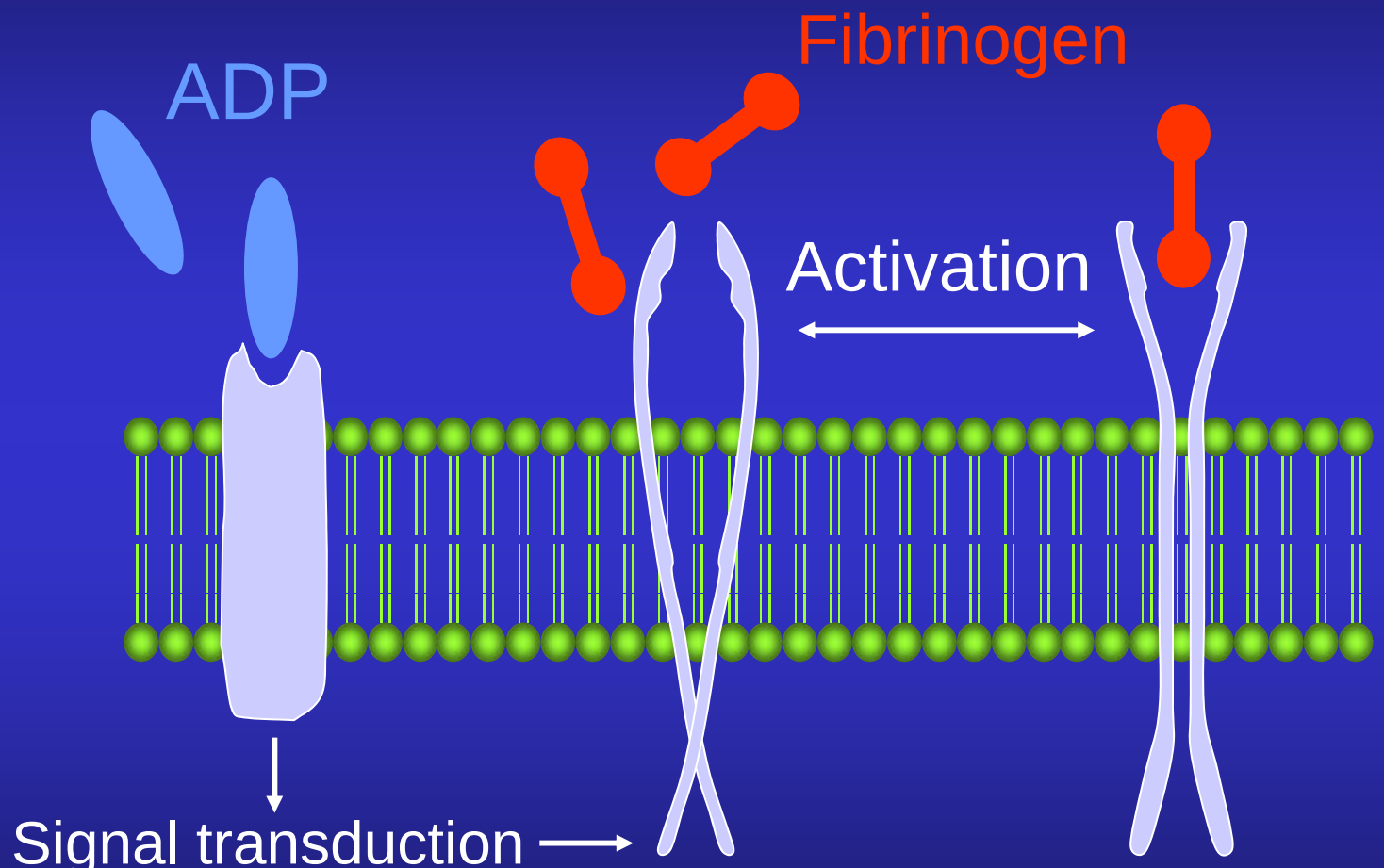
Platelet-Membrane Glycoprotein Receptors Involved in the Adhesion and Aggregation of Platelets

RECEPTOR	LIGAND	ACTION	AA SEQUENCE RECOGNIZED
Integrin			
α2β1 (Gp Ia/IIa)	Collagen	Adhesion	DGEA*
α5β1 (Gp Ic/IIa)	Fibronectin	Adhesion	RGD
α6β1	Laminin	Adhesion	Not confined to a SS
αIIbβ3	Fibrinogen	Aggregation	KQAGDV or RGD
(Gp IIb/IIIa)	Fibronectin		RGD*
	vWF		RGD
	Vitronectin		RGD
αvβ3	Vitronectin	Adhesion	RGD
	Fibrinogen		RGD
	Fibronectin		RGD
	vWF		RGD
Non-integrin			
Gp Ib	vWF	Adhesion	Not confined to a SS
Gp IV	Thrombospondin	Adhesion	CSVTCG
	Collagen		?

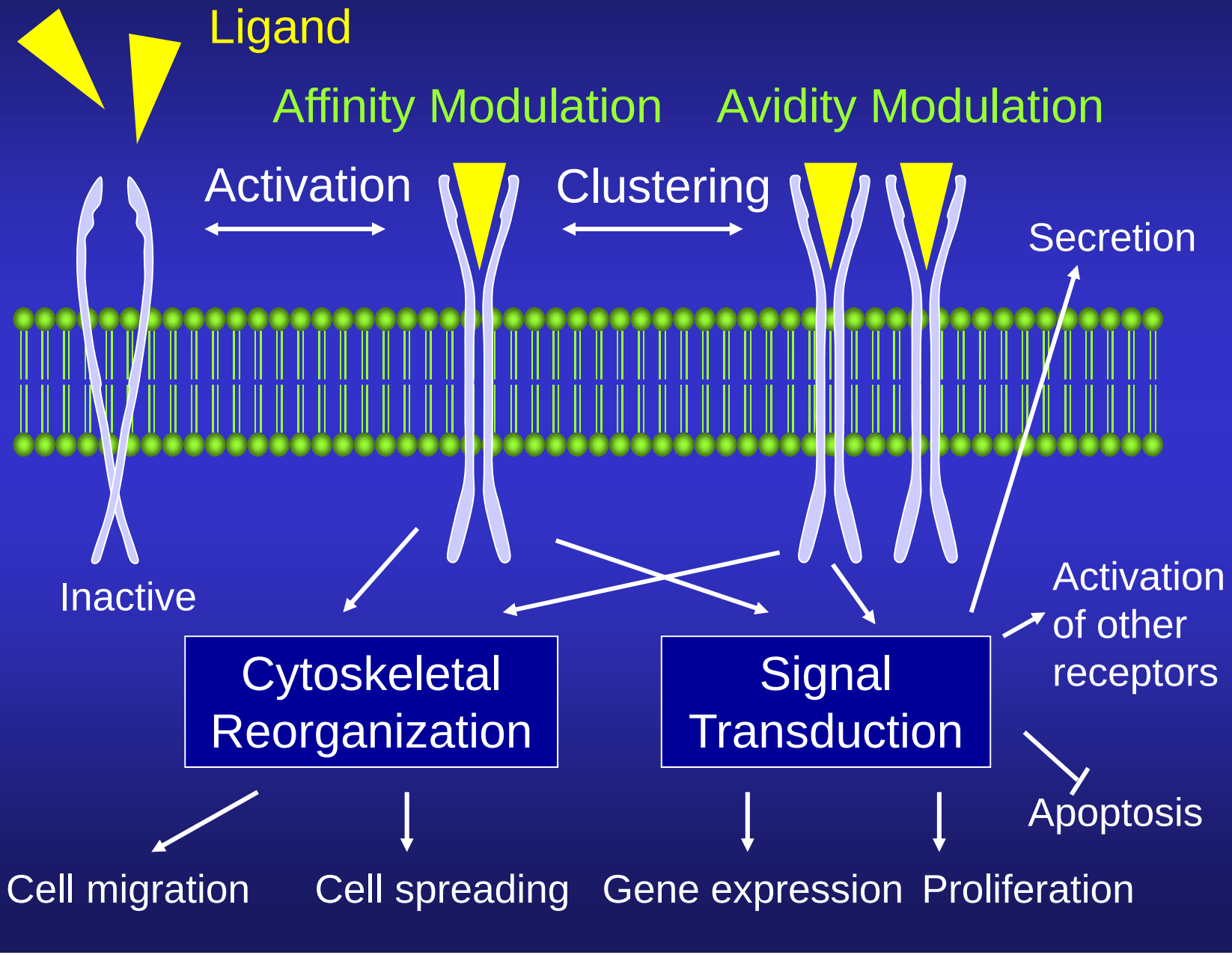
* Other amino acid sequence may also be involved: SS; short sequence

NEJM 1995;332:1553

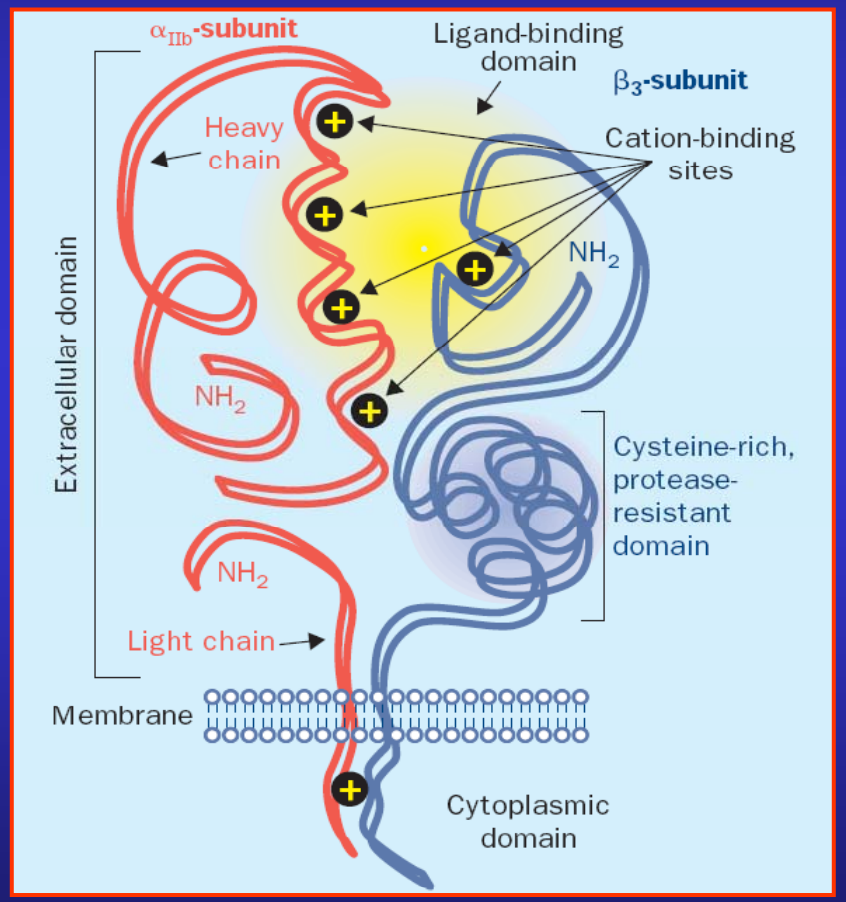
Integrin; “Inside out” signaling

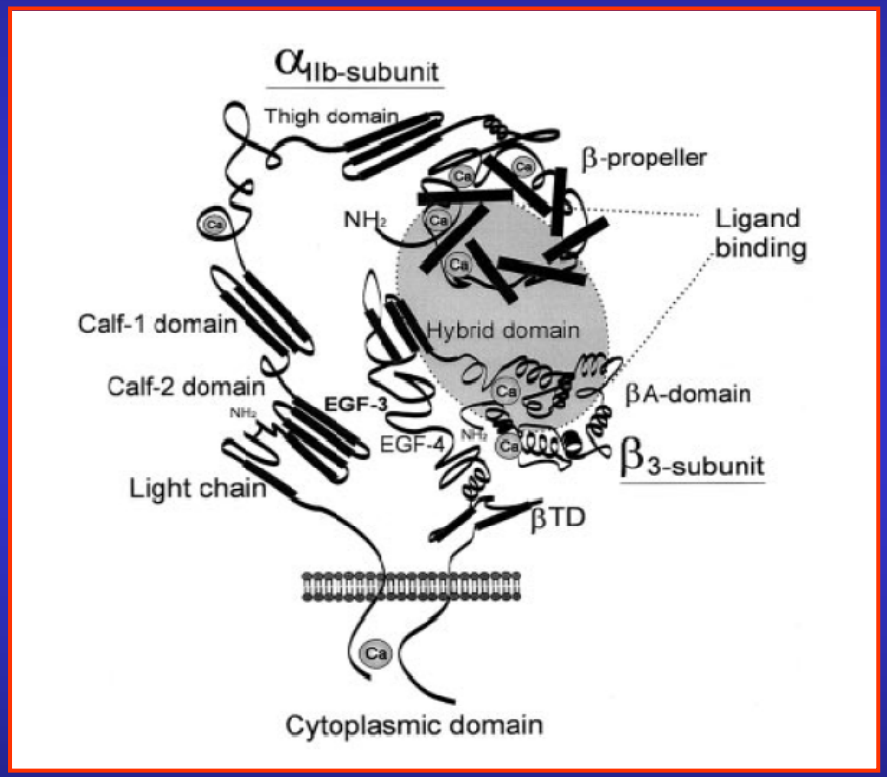


Integrin; “Outside in” signaling



Structure of Integrin $\alpha_{IIb}\beta_3$

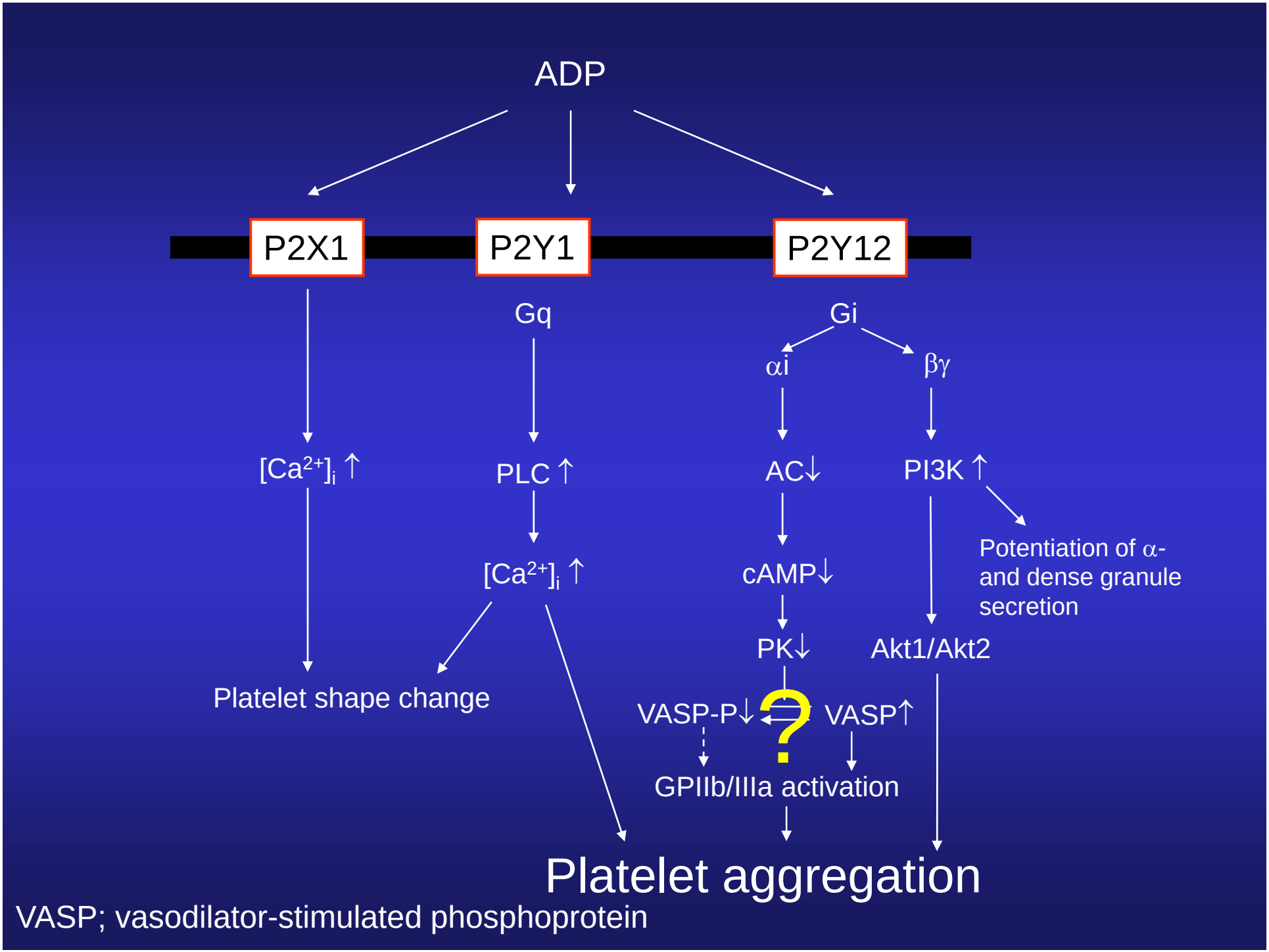




Topol et al, Lancet 1999;353:227-31.; Quinn et al, ATVB 2003;23:945-52.

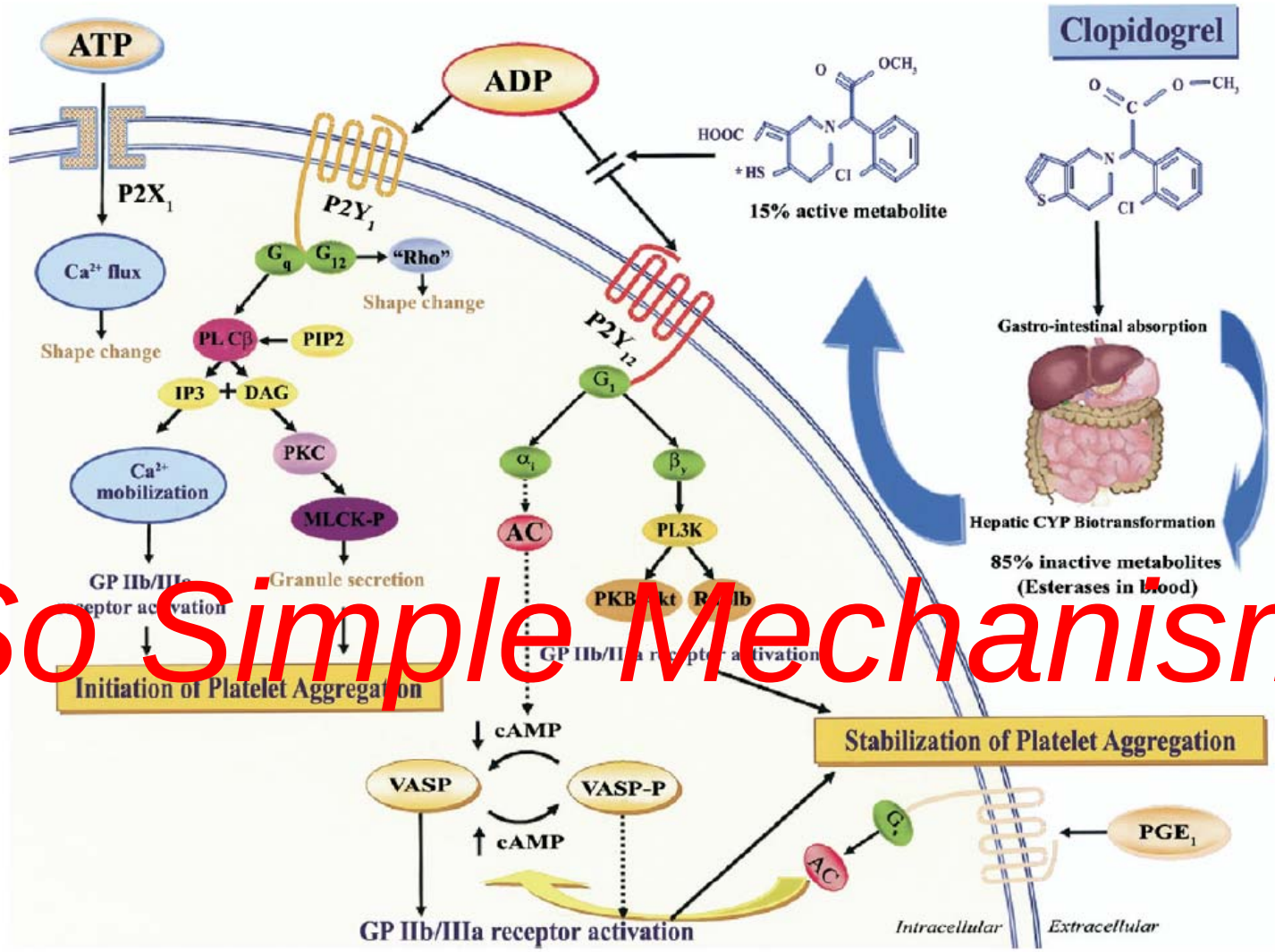
ADP

- Stored at high concentrations in dense granules of platelets, and released on platelet activation.
- Released ADP strongly activates platelets in an autocrine and paracrine fashion.
- It can also be released from damaged cells at places of vascular injury.
- Platelet activation by ADP is mediated by 2 G protein-coupled receptors, P2Y1 (G_q) and P2Y12 (G_{i2}).



VASP; vasodilator-stimulated phosphoprotein

P2 Receptors and Mechanism of Action of Clopidogrel



So Simple Mechanism !

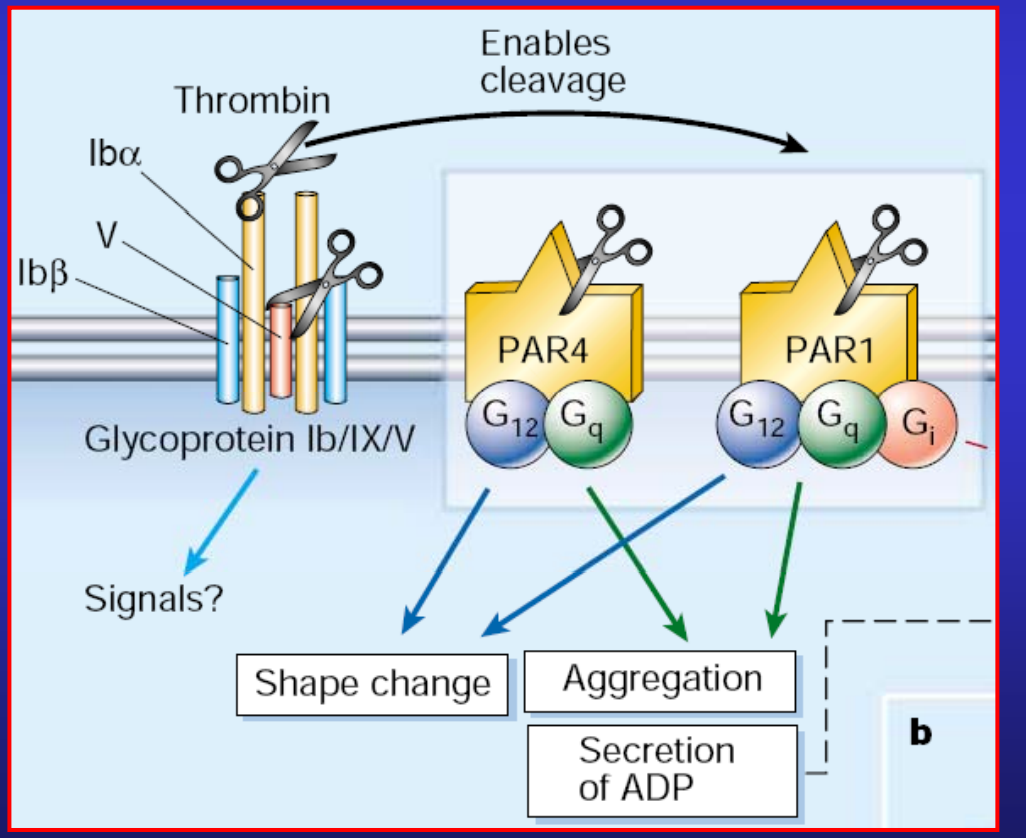
Angiolillo DJ et al, J Am Coll Cardiol 2007;49:1505–16

Thrombin

- Thrombin formation after disruption of the vascular endothelium. Thrombin formation takes place on cellular surfaces including that of activated platelets.
- Protease-activated receptors (PARs)
  - G protein-coupled receptors
  - PAR1 and PAR4 on human platelets
  - PAR1; at low thrombin concentrations
  - PAR4; only at high thrombin concentrations
- SCH 530348
  - an oral reversible PAR1 antagonist

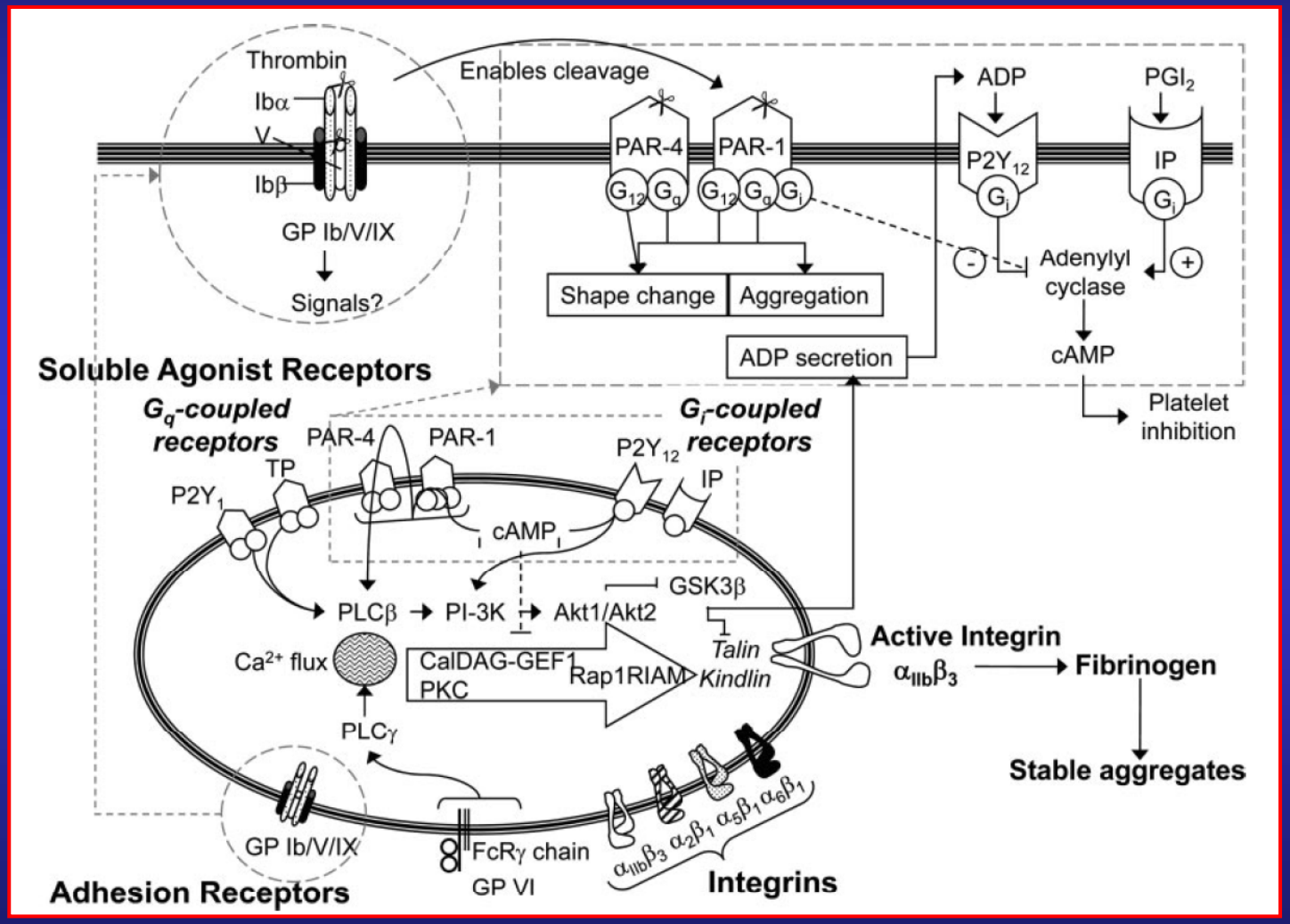
Thrombin; signaling

- Thrombin mediated cleavage of the extracellular domain of the receptor and exposure of a “tethered ligand” at the new end of the receptor
- Signal transduction
  - Activation of PLC and PKC
  - Autoamplification through the production of TXA_2, the release of ADP, and generation of more thrombin on the platelet surface



Brass S. Nature 2001;413:26

Role of G protein–coupled Receptors in the Thrombotic Process

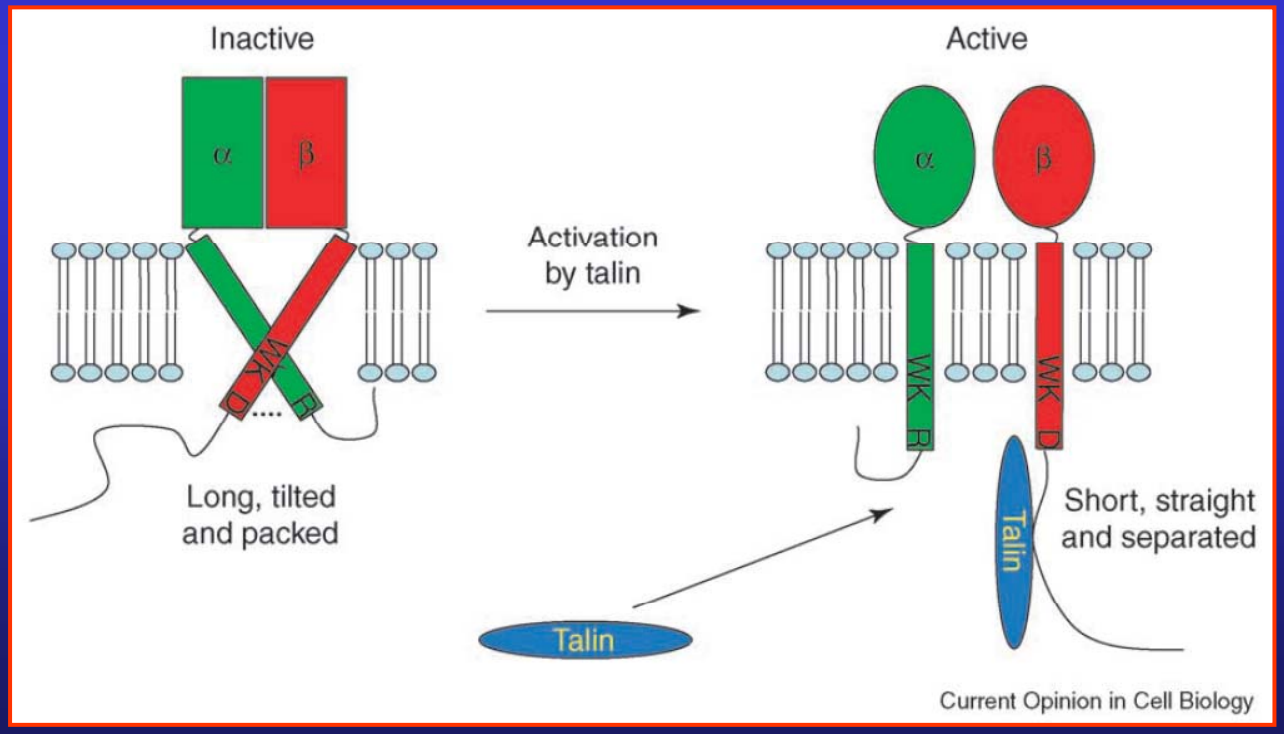


CalDAG-GEF1, calcium and diacylglycerol-regulated guanine-nucleotide exchange factor 1
RIAM, Rap1-GTP–interacting adapter molecule

2008 Platelet Colloquium Participants, ATVB 2009;29:449-457

Model for Talin-mediated Activation of Integrin

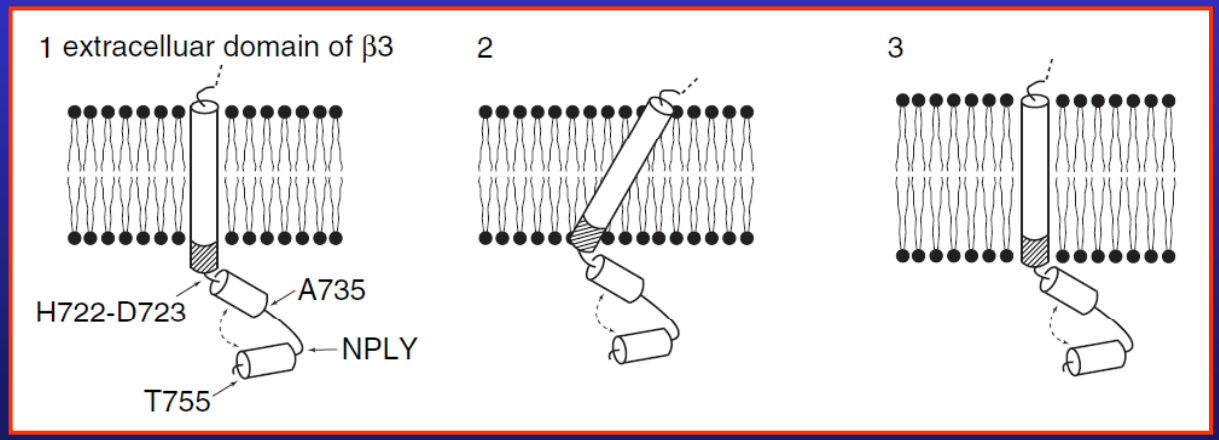
- Low-affinity integrin; inter-subunit interactions
  - TM helices; specifically pack together and in a long and tilted geometry.
  - Membrane-proximal domains; salt bridge (dotted line) between an a subunit arginine (Arg995, R) and β subunit aspartic acid (Asp723, β3, D).
- High affinity integrin after talin binding to β3 tail
  - Breaking of TM helical packing
  - Disruption of the membrane-proximal salt bridge



Ginsberg MH , et al, Curr Opin Cell Biol 2005, 17:509

β3 Cytoplasmic Tail

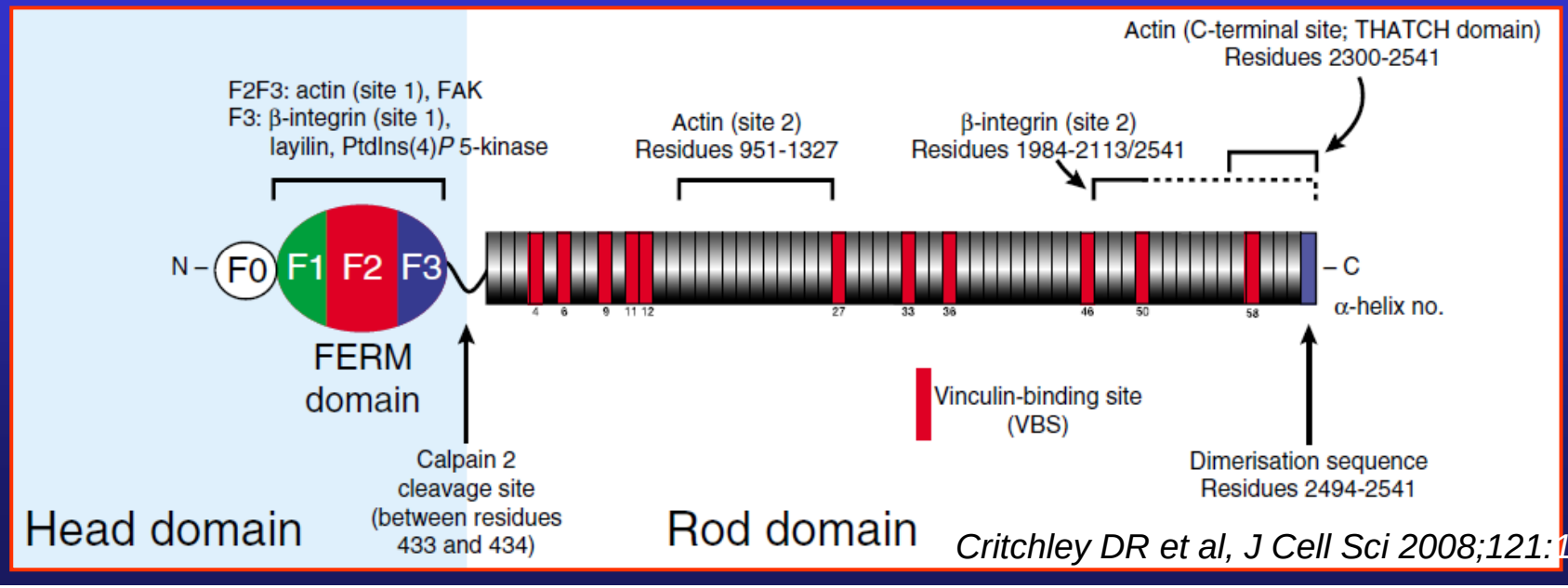
- β3 TM and cytoplasmic domain
- β3 TM helix - hinge - 2nd helix - hinge - NPLY motif - 3rd helix - NITY motif
  - NPLY motif (residues 744-747); talin FERM domain
  - NITY motif (residues 756-759); kindlin-3 FERM domain
- Interaction with large number of cytosolic protein, but identified functional significance in a few proteins
  - Talin-1, Kindlin-3, Rap1b/CalDAG-GEFI, RIAM



Bennett JS et al, J Thromb Haemost, 2009;7:200

Talin-1

- A 270-kDa cytoplasmic protein
- Binding to both β cytoplasmic tail and actin
- Head domain + rod domain
- FERM domain (pretein 4.1, ezrin, radixin, moesin)
- F1, F2, F3 subdomain
- F3 subdomain contains a phosphotyrosine binding (PTB) domain, which interacts with the conserved NPLY motif of β tails



Critchley DR et al, J Cell Sci 2008;121:1345

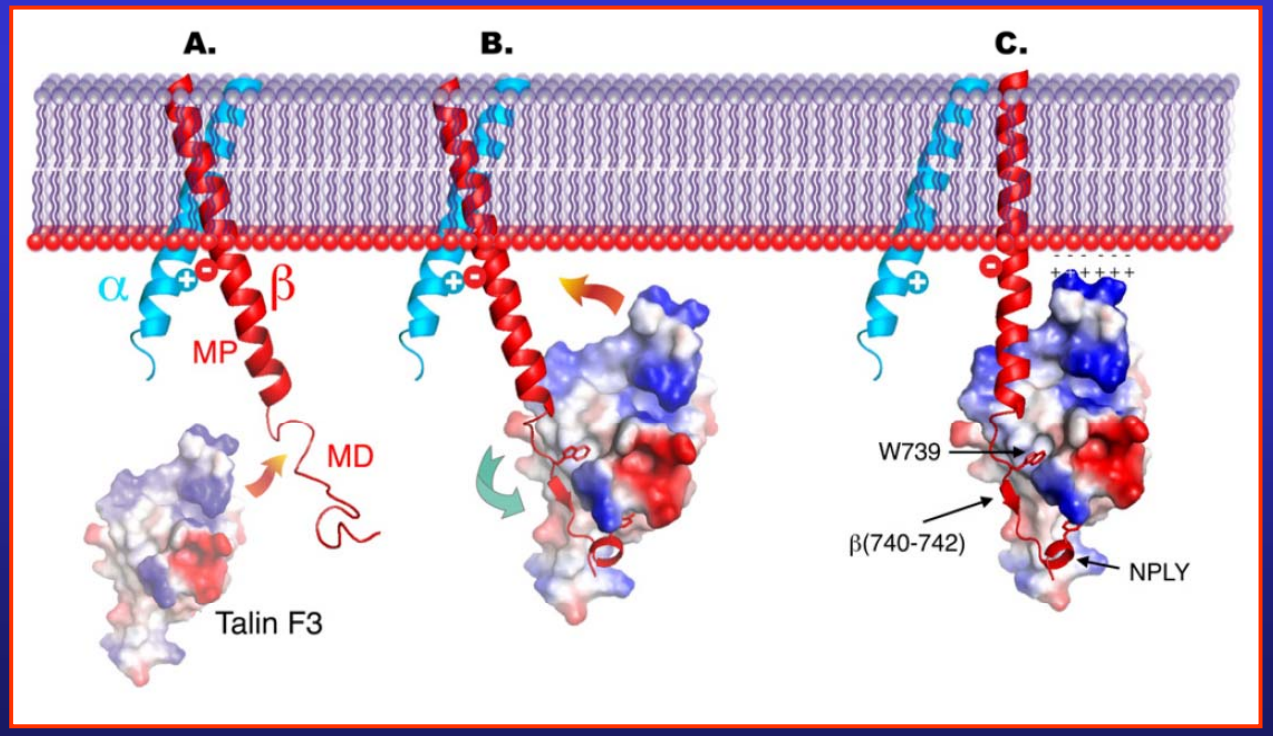
Talin-1

Conditional talin-1-deficient mice (*Tln*$^{-/-}$, CreloxP)

- defective hemostasis and abrogated platelet adhesion, and thrombus formation in injured vessels *in vivo*

Talin-1-deficient platelets

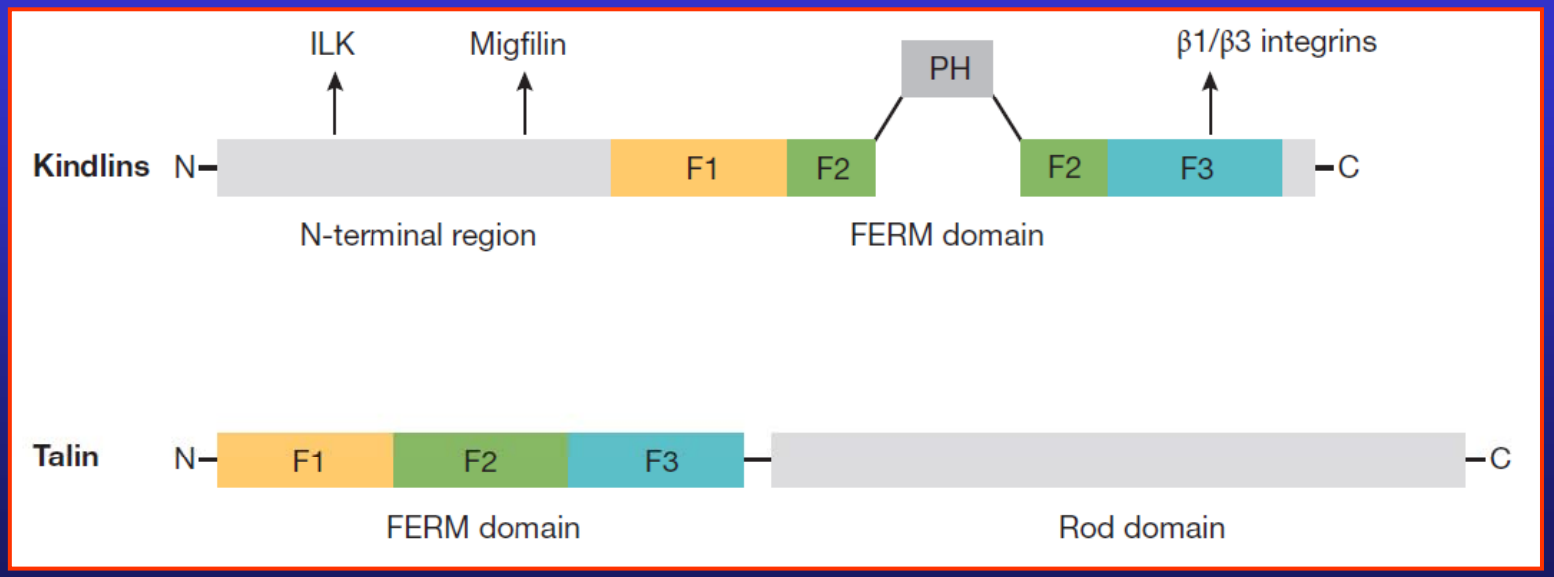
- unable to activate integrin αIIβ3 or aggregate
- fail to spread on immobilized fibrinogen, suggesting that talin-1 is also required for αIIβ3-dependent outside-in signaling



Wegener KL et al, Cell 2007;128:171–182

Kindlin Family

- 3 mammalian isoforms
  - kindlin-1 (also known as kindlerin and FERMT1)
  - kindlin-2 (also known as MIG -2)
  - kindlin-3 (also known as URP 2)
  - identical domain architecture and high sequence similarities
- Highly concentrated at sites of cell-ECM adhesion
- 4 kindlin binding proteins
  ILK, migfilin, β1-integrin and β3-integrin
- Crucial components of cell-ECM adhesion



*Larjava H et al, EMBO reports 2008;**9:1203–1208***

Kindlin-3

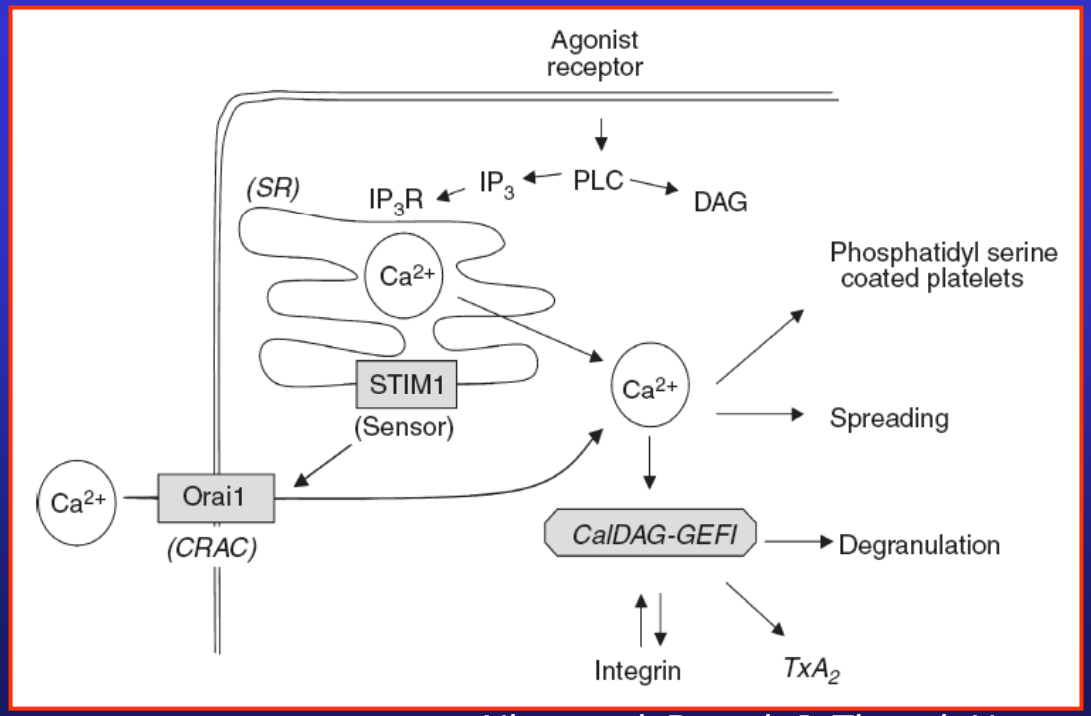
- The germ line knockout of kindlin-3
  perinatal lethality associated with diffuse hemorrhages
- Fetal liver cell chimeric kindlin-3$^{-/-}$ mice
  severe hemostatic defect and resistance to arterial thrombosis
- kindlin-3$^{-/-}$ platelets
  - unable to activate integrin αIIbβ3, despite unaltered expression of talin-1
  - did not spread on fibrinogen, suggesting a role in outside-in signaling processes.
- β3 cytoplasmic tail
  - NPLY motif (residues 744-747); talin FERM domain
  - NITY motif (residues 756-759); kindlin-3 FERM domain

Nieswandt B et al, J Thromb Haemost 2009;7 (Suppl 1): 206–209

Platelets Calcium Signaling

Cytoplasmic Ca^{2+} transient after agonist stimulation

- Intracellular source of Ca^{2+}
  - Transient mobilization of limited Ca^{2+} amounts released from SR
- Store-operated Ca^{2+} entry (SOCE)
  - The major mechanism for entry of extracellular Ca^{2+}
  - Stromal interaction molecule 1 (STIM1); SR-resident Ca^{2+} sensor
  - Depletion of SR Ca^{2+} stores triggers the activation of Ca^{2+} release activated calcium (CRAC) channels Orai1 in the plasma membrane



Nieswandt B et al, J Thromb Haemost 2009;7 (Suppl 1): 187

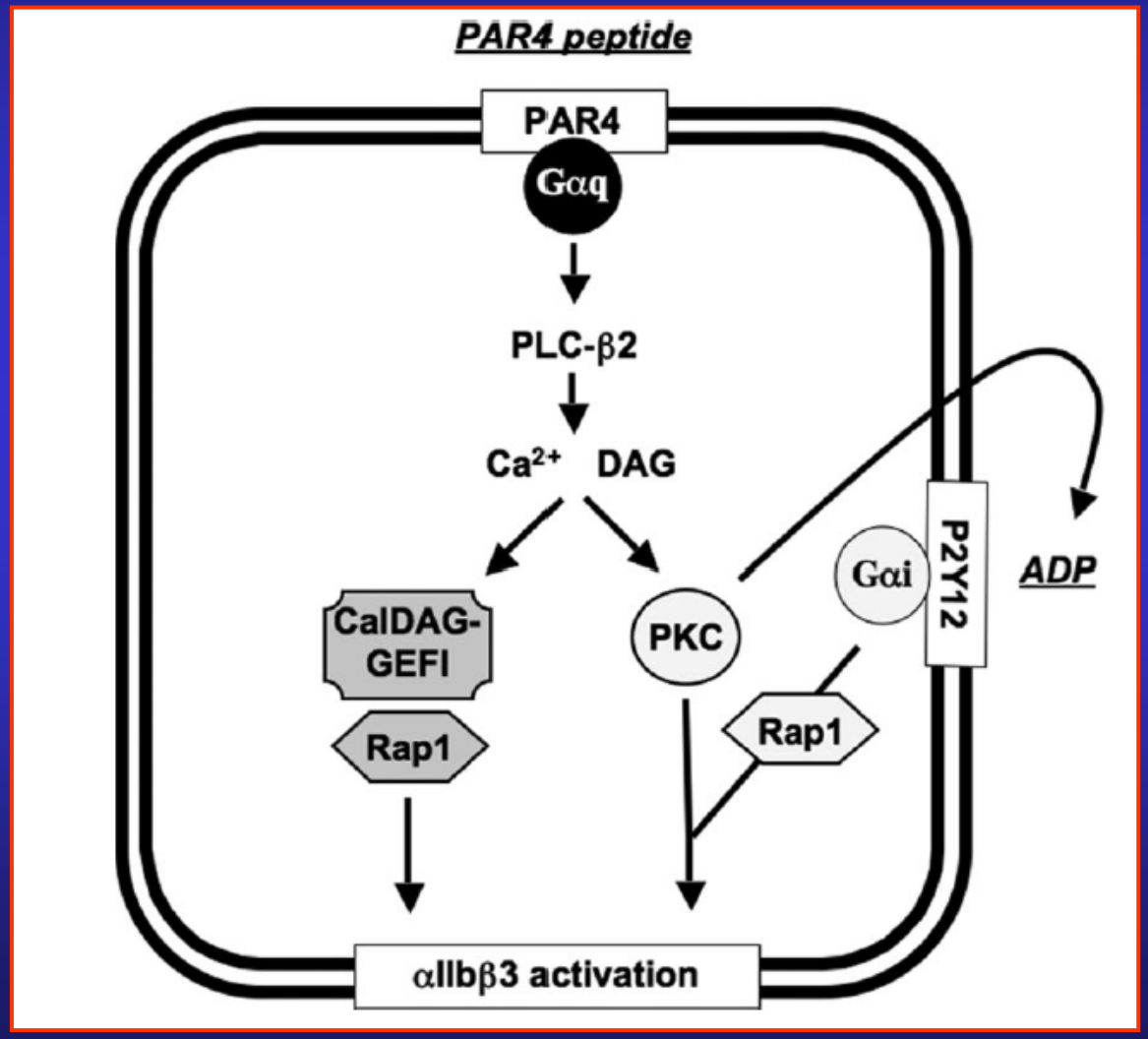
Rap1b/CalDAG-GEFI

- Rap1b is a small GTP binding protein of the Ras family.
- Deficiency of Rap1b in platelets leads to defective $\alpha IIb\beta 3$ activation, prolonged bleeding times, and protection against arterial thrombosis.
- Activation of Rap1b is controlled by
  1. CalDAG-GEFI; rapid but reversible Rap1 activation
  2. Protein kinase C (PKC); sustained Rap1 activation
  - ❖ Cal-DAG-GEFI deficiency
    - impaired platelet aggregation responses to ADP or TxA2 ex vivo
    - prolonged bleeding times and protection from arterial thrombosis in vivo.

CalDAG-GEFI; Ca^{2+} and diacylglycerol-regulated guanine-nucleotide-exchange factor I

Nieswandt B et al, J Thromb Haemost 2009;7 (Suppl 1): 206–209

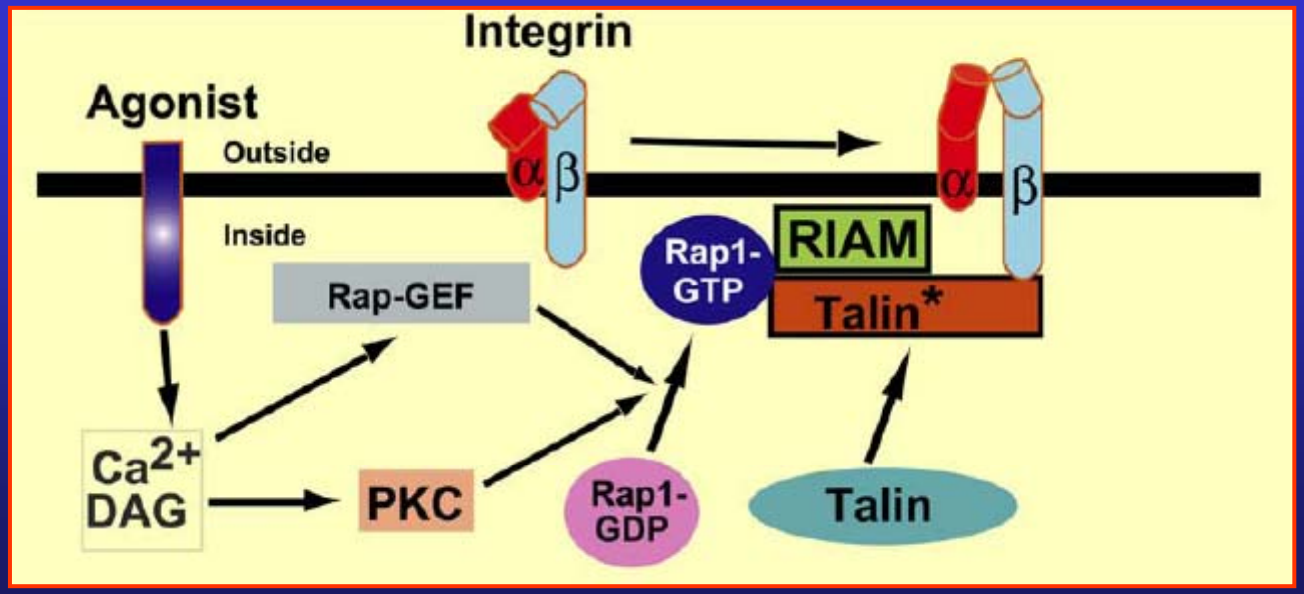
Schematic representation of the CalDAG-GEFI-dependent and PKC-dependent signaling pathways leading to αIIβ3 activation in mouse platelets



Cifuni SM et al, Blood 2008;112:1696-1703

RIAM (Rap1-GTP-interacting adaptor molecule)

- Rap1 effector molecule
- A member of MRL family of adaptor molecules (Mig-10, RIAM, and lamellipodin)
- Interaction with both Rap1-GTP and talin-1
- Knock down; blocks talin-1 recruitment to αIIβ3 and integrin activation
- Over-expression; integrin activation and enhanced cell adhesion
- Rap1-induced formation of *an integrin activation complex* consisting of RIAM and talin-1 that leads to the unmasking of the integrin-binding site on talin-1



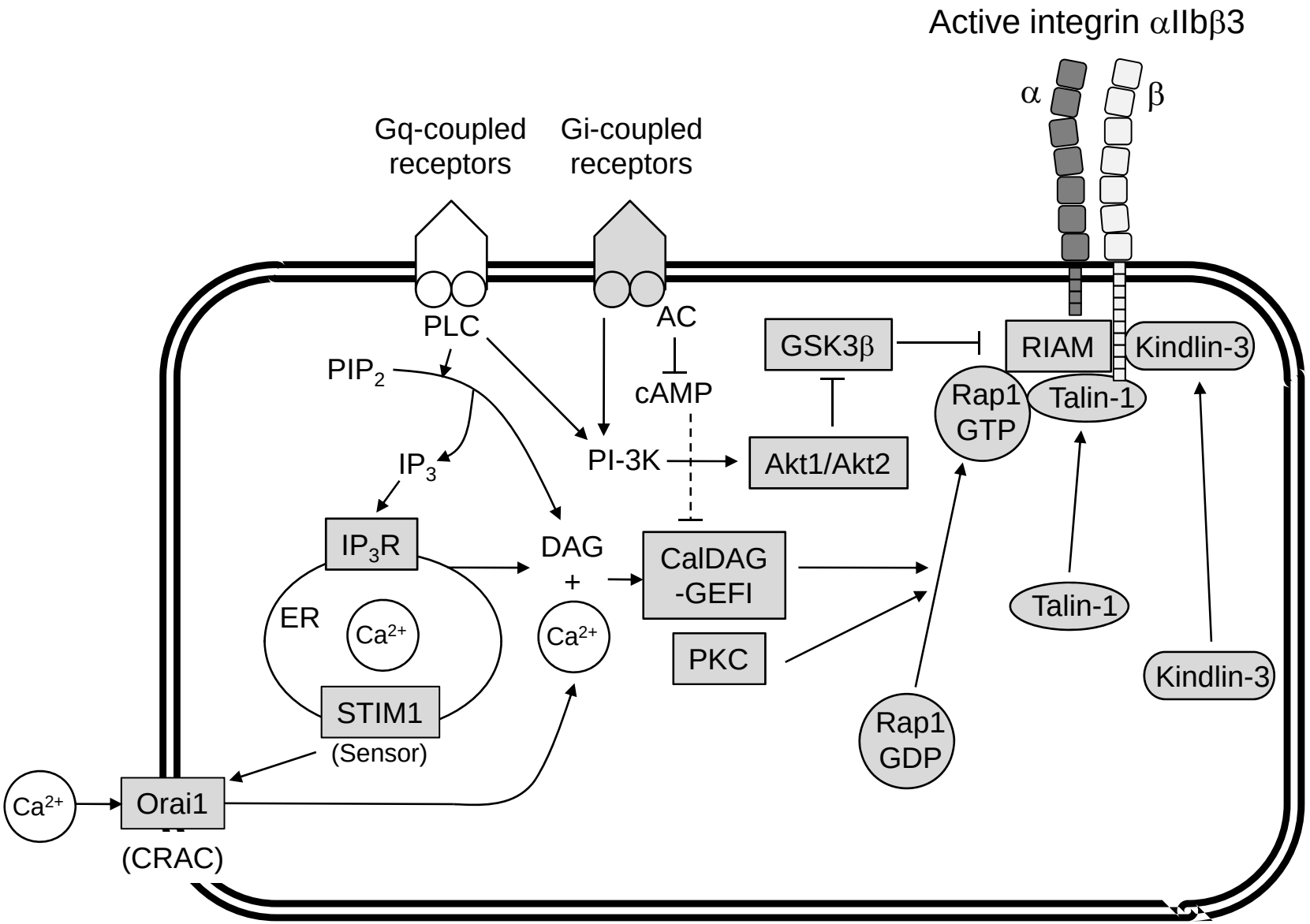
Han J et al, Curr Biol 2006;16:1796

Protein Kinase Akt

- A principal target for PI-3K signaling
- Both Akt1 and Akt2 isoforms in platelets.
- Both Akt1 and Akt2 are required for thrombus formation in mice
- Glycogen synthase kinase (GSK)-3 suppresses platelet function and thrombosis in mice
- Akt mediated phosphorylation of GSK-3 inhibits the kinase activity of the enzyme, and with it, its suppression of platelet function

Smyth SS et al, ATVB 2009;29:449

Summary


Active integrin αIIbβ3
α
β
Gq-coupled receptors
Gi-coupled receptors
PLC
AC
PIP2
cAMP
GSK3β
RIAM
Kindlin-3
Rap1 GTP
Talin-1
IP3
PI-3K
Akt1/Akt2
IP3R
DAG
+
CalDAG -GEFI
ER
Ca2+
Ca2+
PKC
Talin-1
Kindlin-3
STIM1
(Sensor)
Rap1 GDP
Ca2+
Orai1
(CRAC)