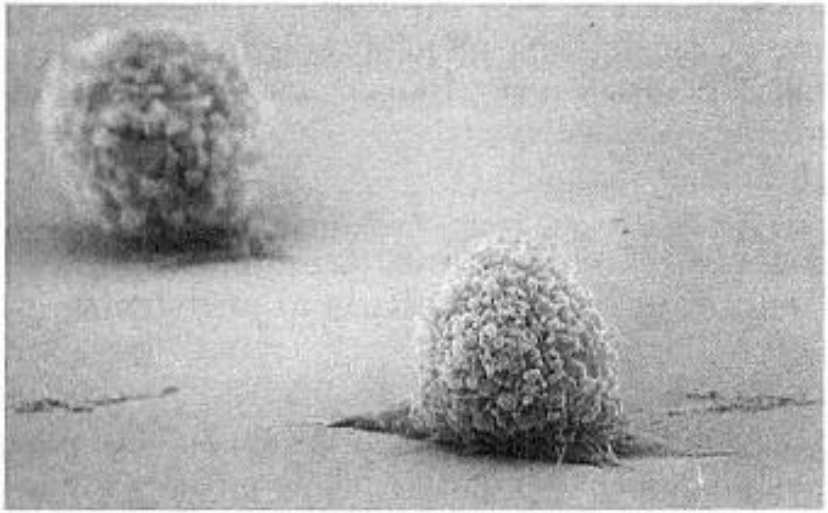
Adesione cellulare:

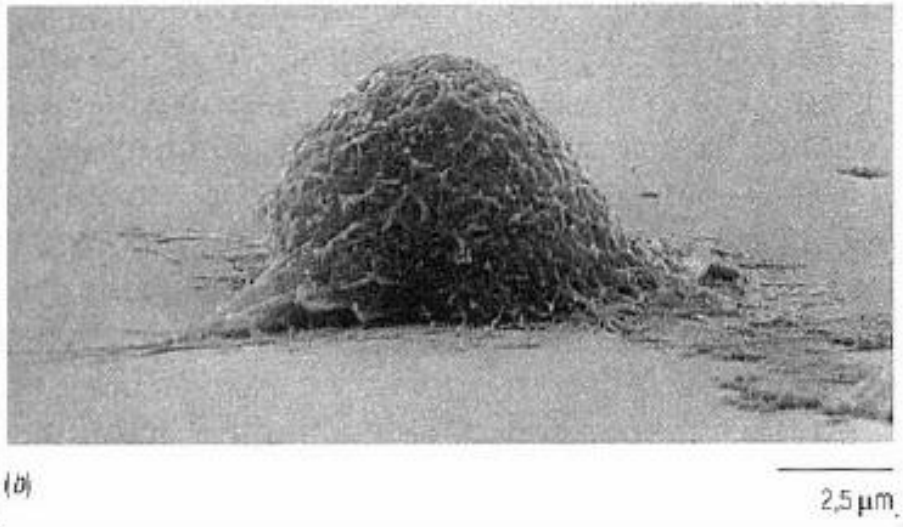
Responsabile della segregazione e dell'architettura dei tessuti

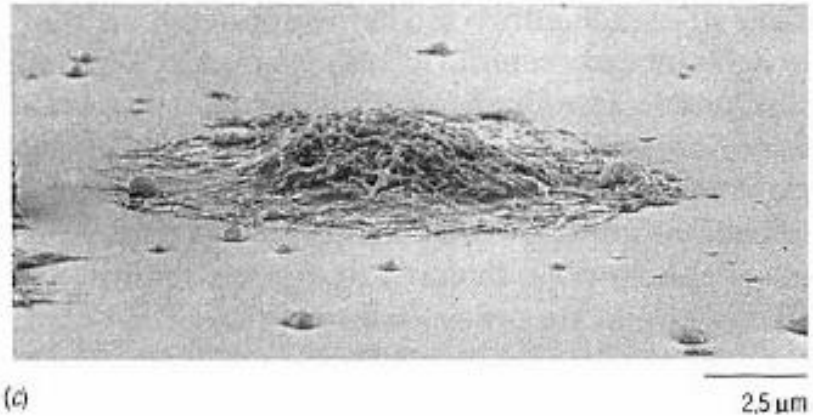
Interazioni che permettono alle cellule di aderire tra loro, interconnettono i citoscheletri di cellule adiacenti e conferiscono rigidità a resistenza ai tessuti

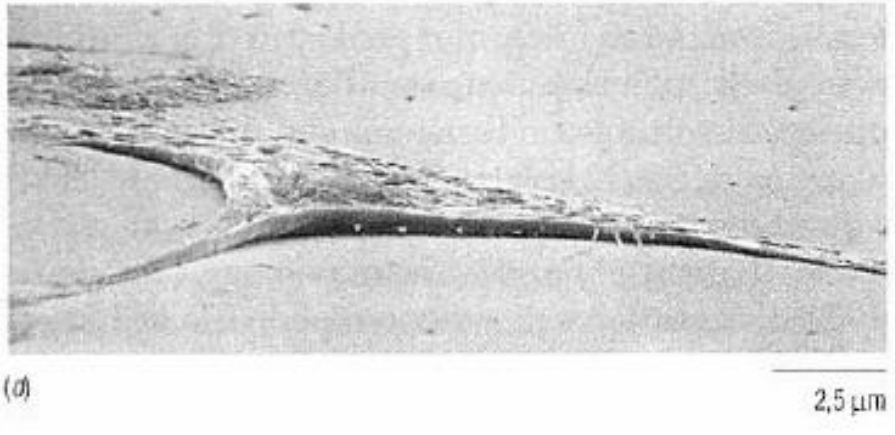
Morfogenesi crescita divisione morte

cellula osteoblastica durante il processo di adesione





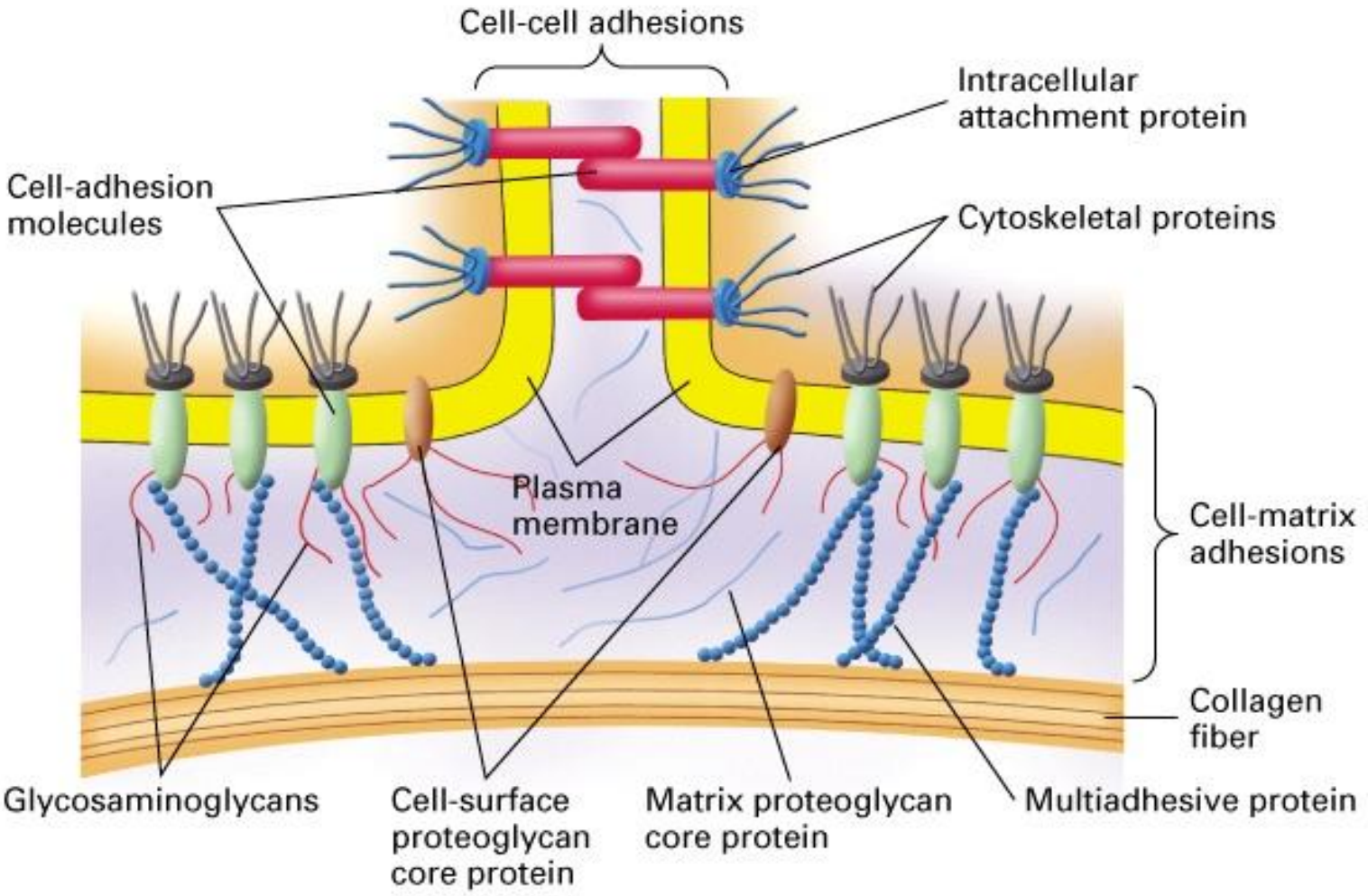


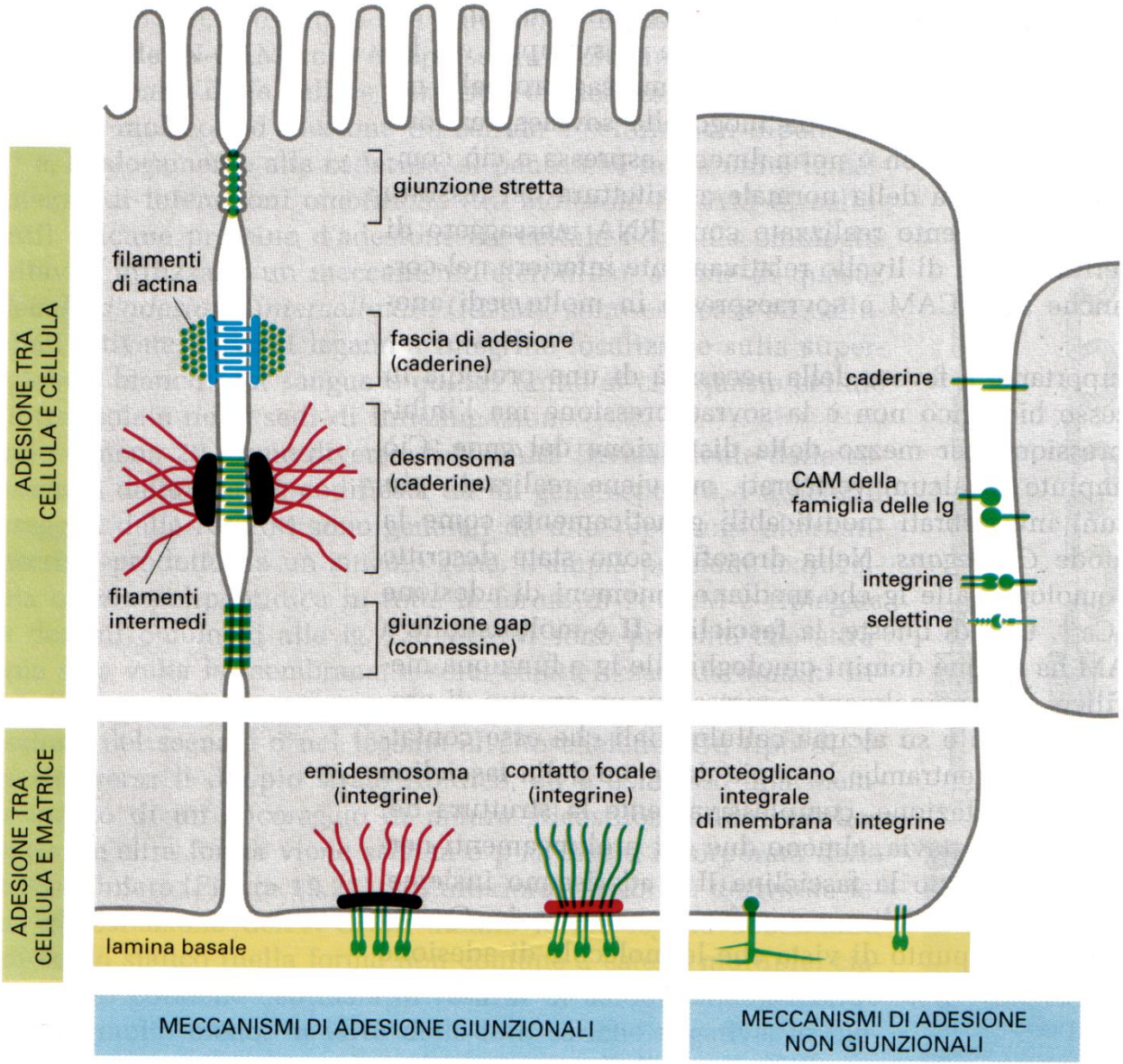


Meccanismi di adesione cellulare

Adesioni cell-cell

Adesioni cell-matrice




ADESIONE TRA CELLULA E CELLULA
giunzione stretta
filamenti di actina
fascia di adesione (caderine)
desmosoma (caderine)
filamenti intermedi
giunzione gap (connessine)
caderine
CAM della famiglia delle Ig
integrine
selettine
ADESIONE TRA CELLULA E MATRICE
emidesmosoma (integrine)
contatto focale (integrine)
lamina basale
proteoglicano integrale di membrana
integrine
MECCANISMI DI ADESIONE GIUNZIONALI
MECCANISMI DI ADESIONE NON GIUNZIONALI

CAM molecole di adesione cellulare

proteine integrali di membrana che permettono alle cellule di aderire e interagire con l'esterno

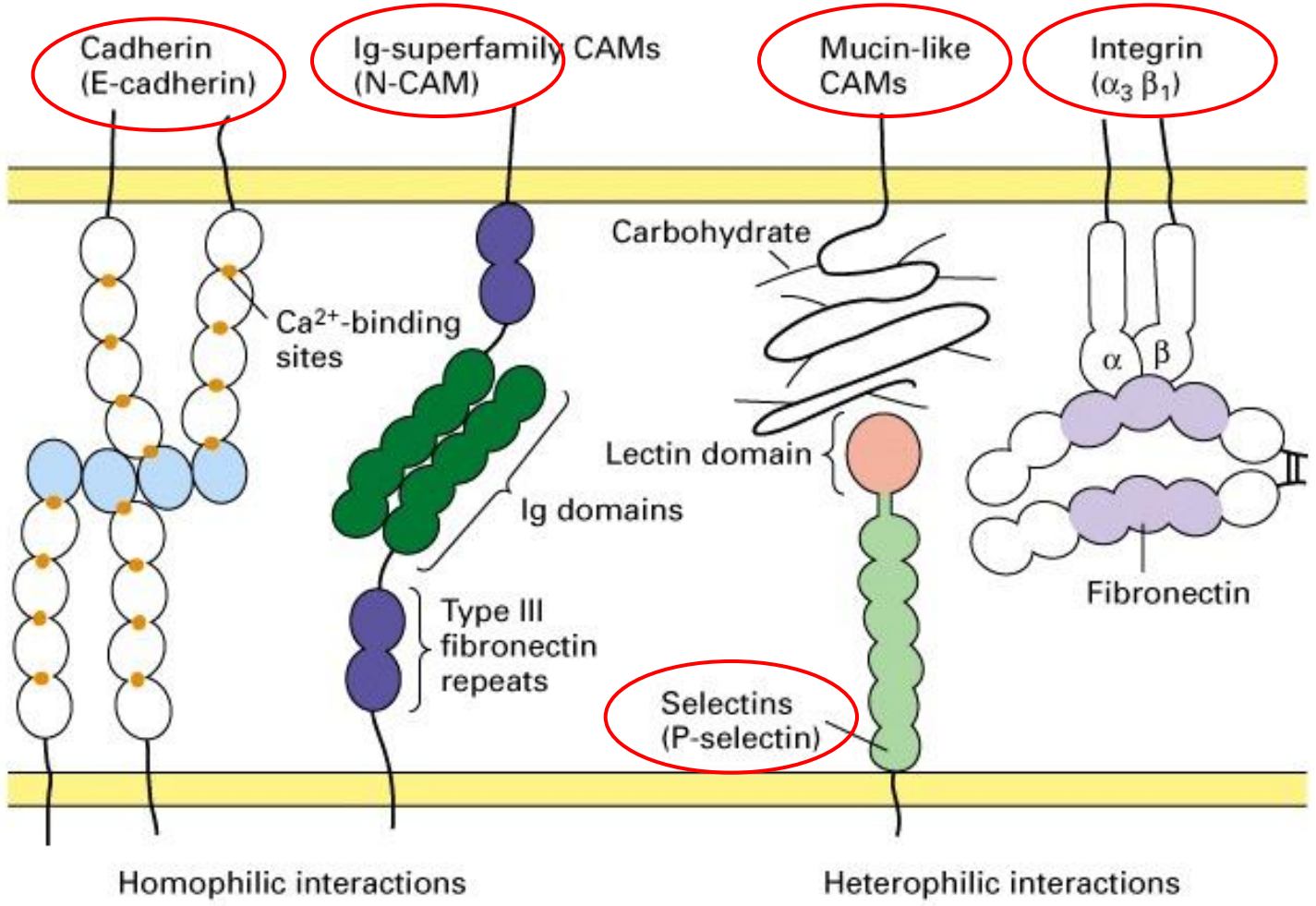
ECM matrice extracellulare

complessa rete di carboidrati e proteine secreti dalle cellule animali, ruolo strutturale (divisione e movimento) e funzionale (ormoni)

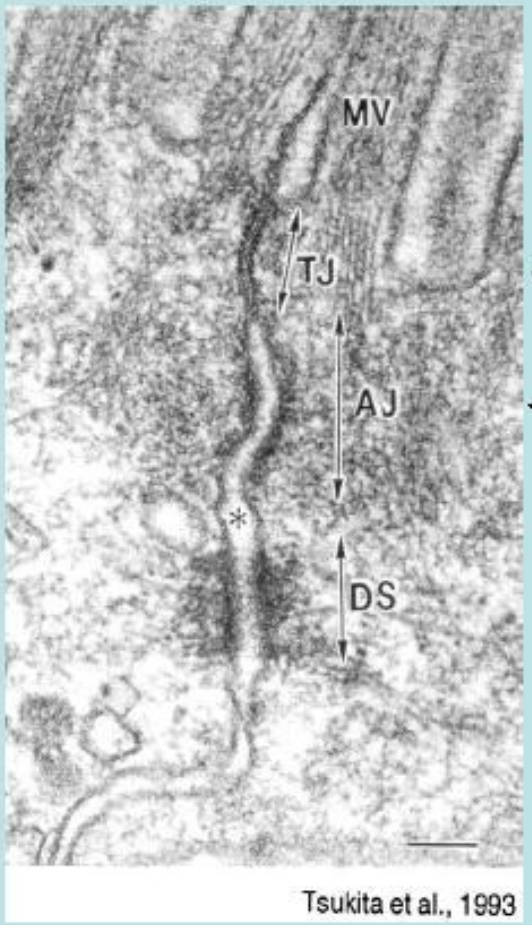
Giunzioni

raggruppamenti di CAM

Esistono 5 classi principali di molecole di adesione cellulare (CAMs)



Giunzioni:



villi

tight junctions

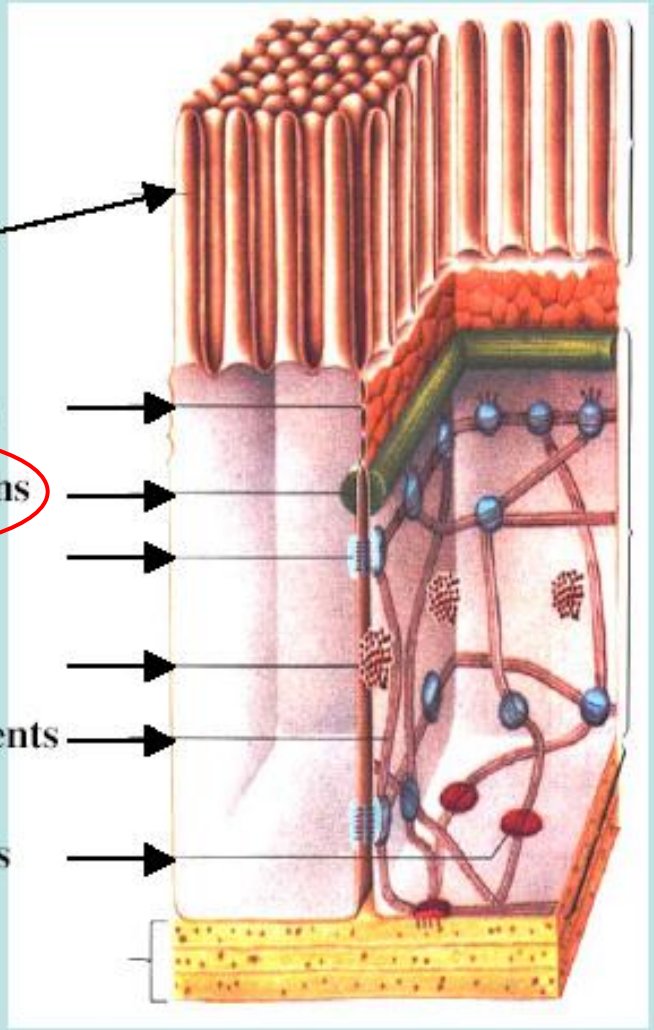
adherens junctions

desmosomes

gap junctions

actin / inter. filaments

emi-desmosomes



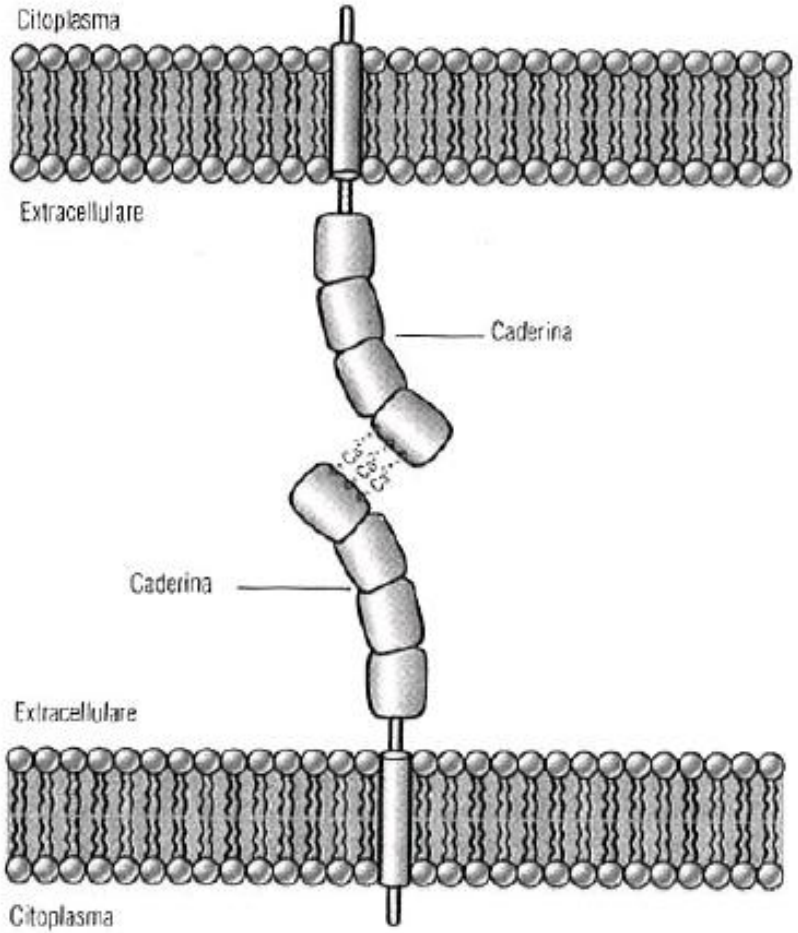
CAM proteine di connessione filamenti del citoscheletro

Giunzioni aderenti: Caderine

- 40 tipi diversi, subfamiglie
- Ruolo cruciale durante il differenziamento
- “classiche”:

E-caderina	embrione, cell epiteliali
N-caderina	sistema nervoso
P-caderina	cuore, polmoni, intestino

Caderine



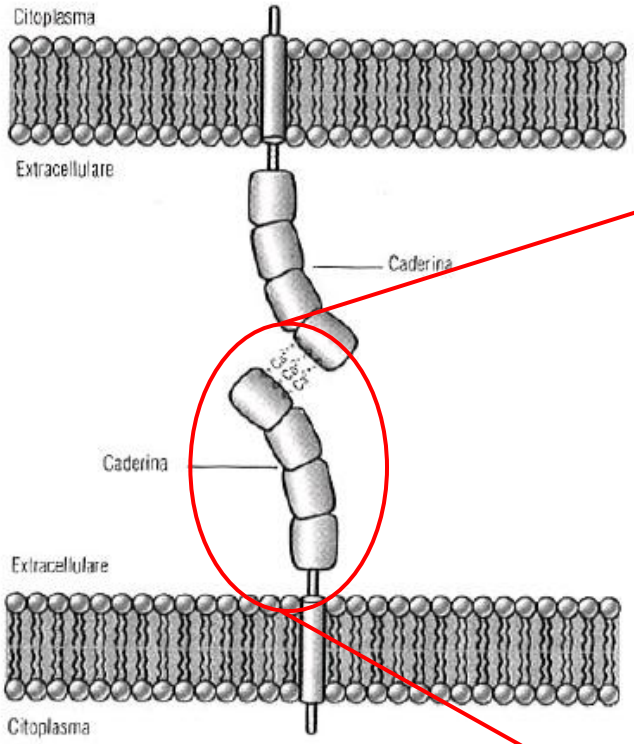
720-750 aa

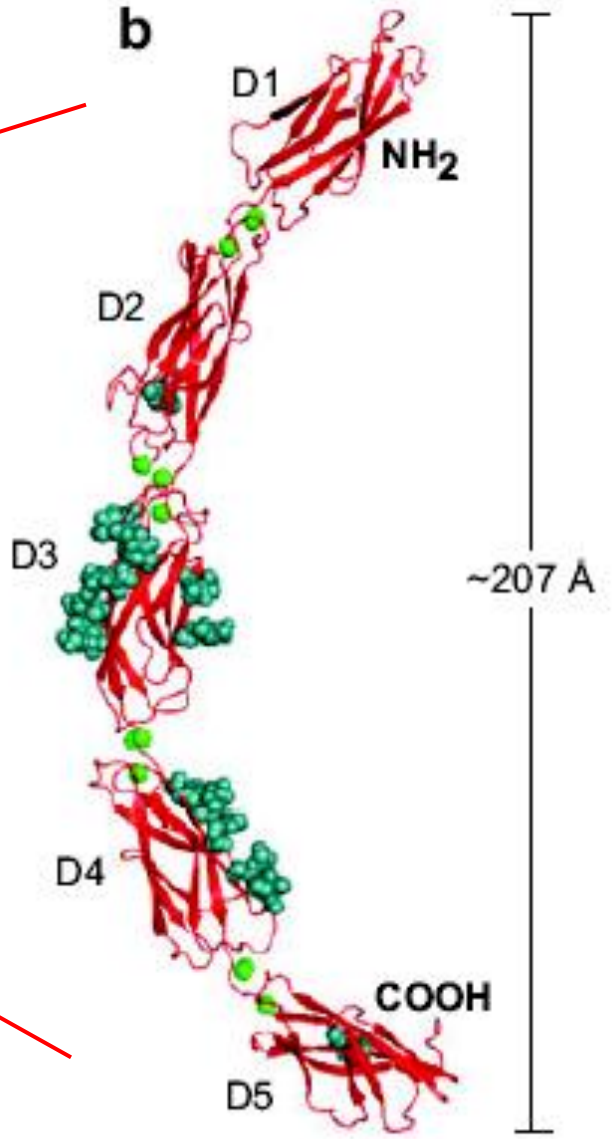
•Ectodominio, N-term extracellulare

•Un segmento transmembrana

•C-term intracellulare

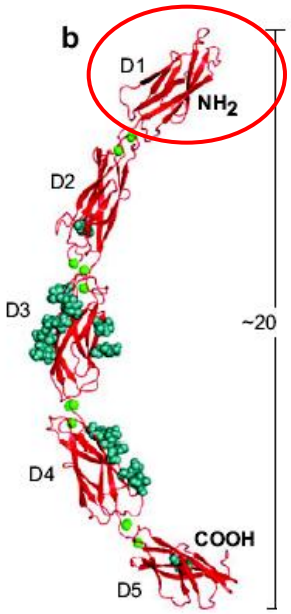
Caderine “classiche”: C-caderina

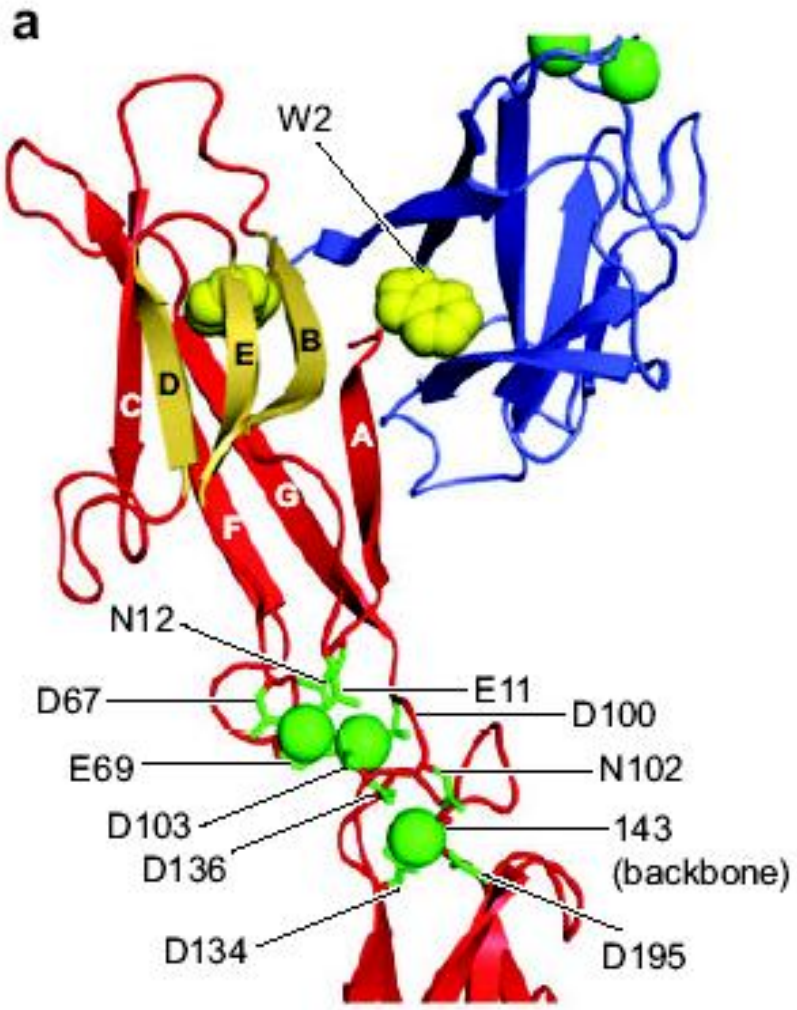




- 5 EC/D
- Ca^{2+} dipendenti
- N- O-glicosilazioni

D1:





7 filamenti beta

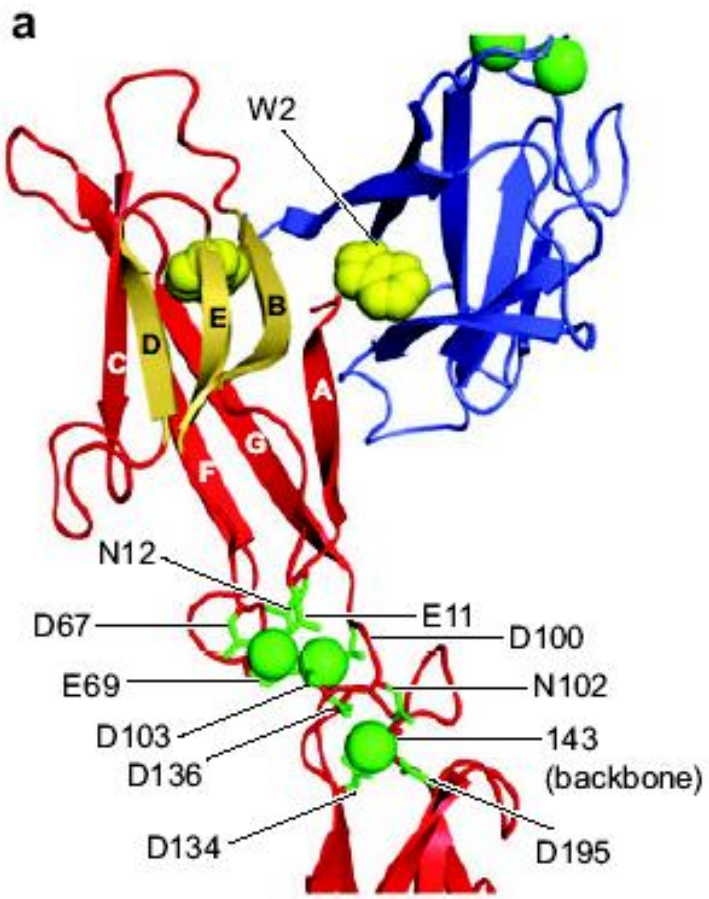
↓

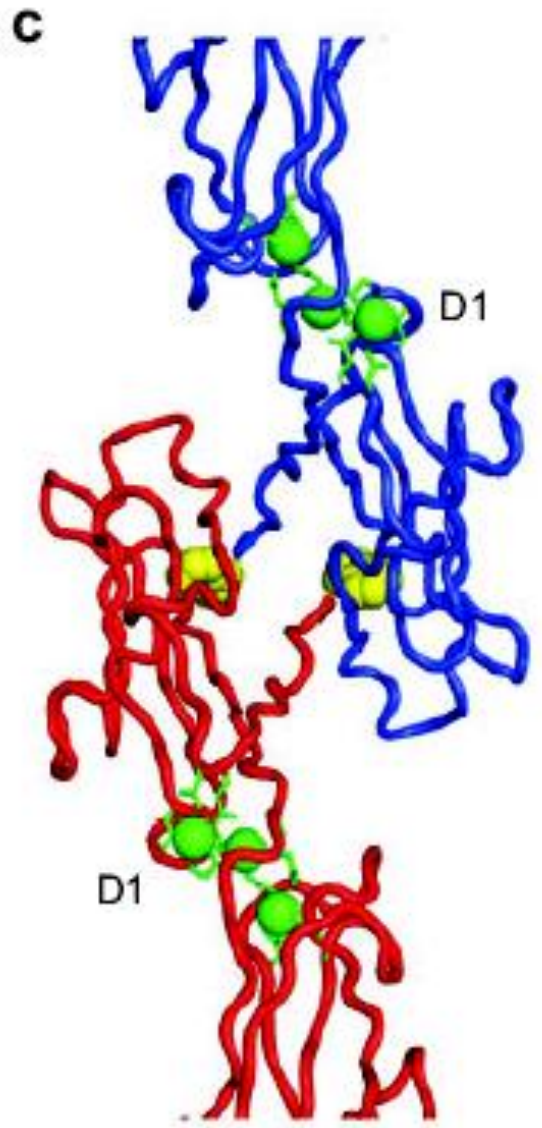
2 foglietti beta

↓

beta sandwich

Legame caderina - caderina: D1-D1

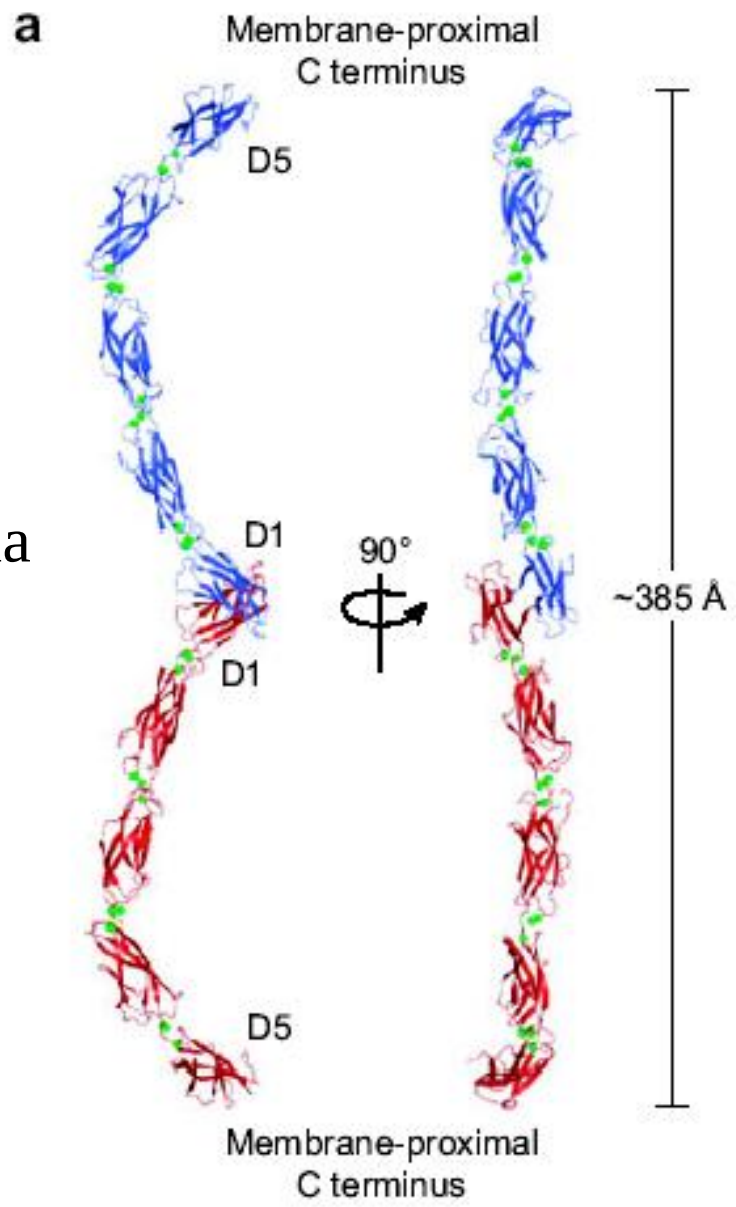




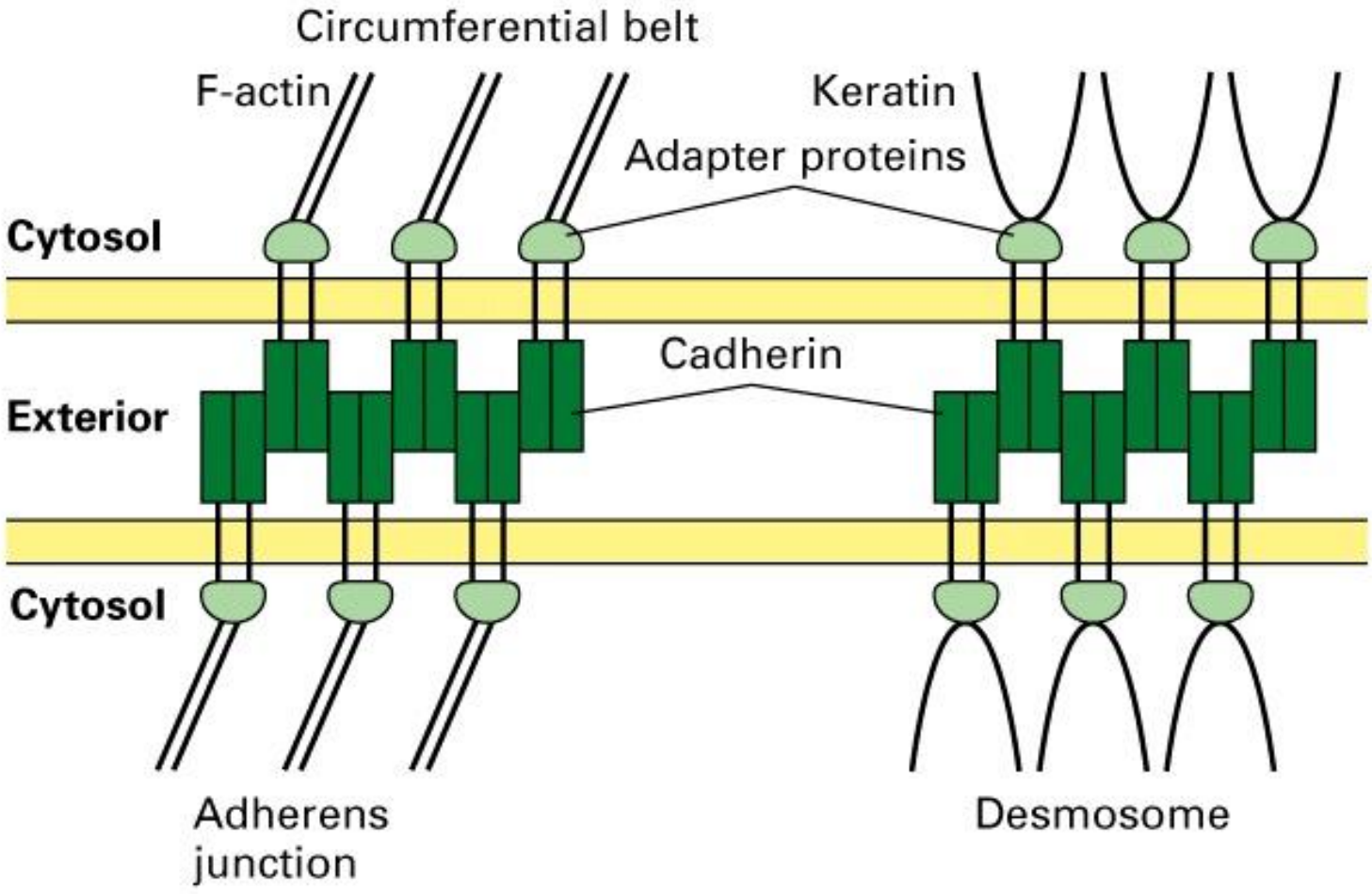
- Scambio filamento A
- Trp2

Legame caderina - caderina: D1-D1

Interazione parallela



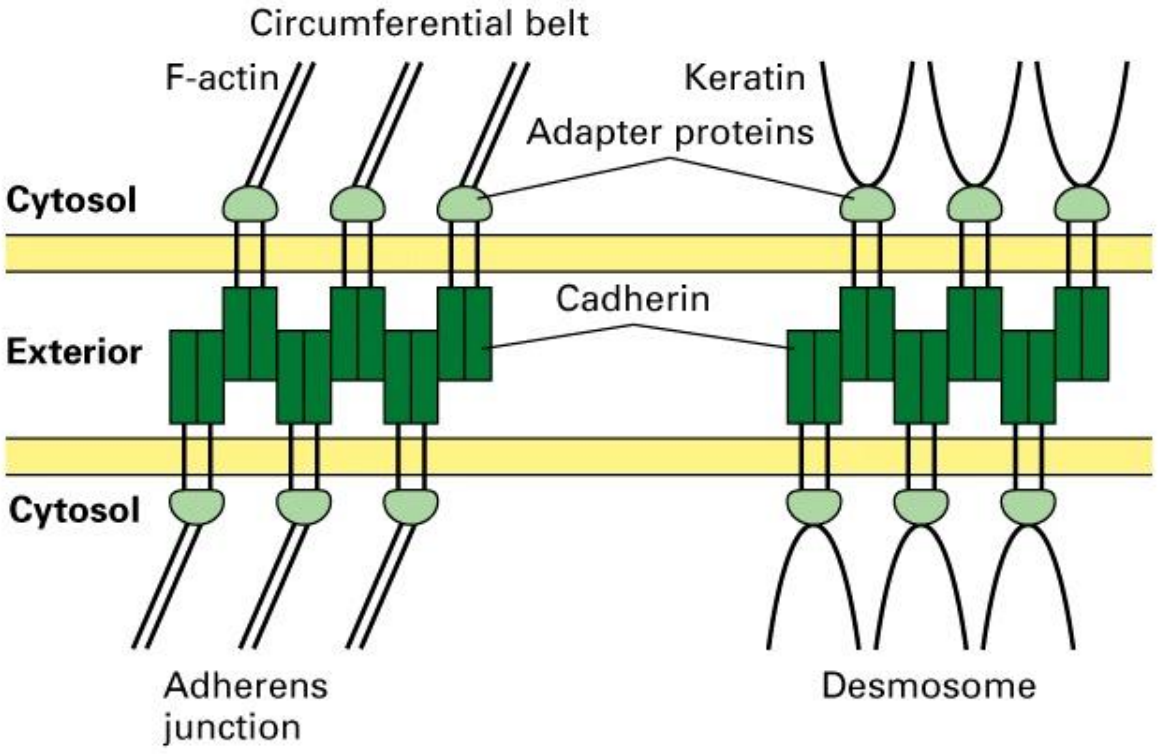
Giunzioni mediate da caderine



Giunzioni mediate da caderine:

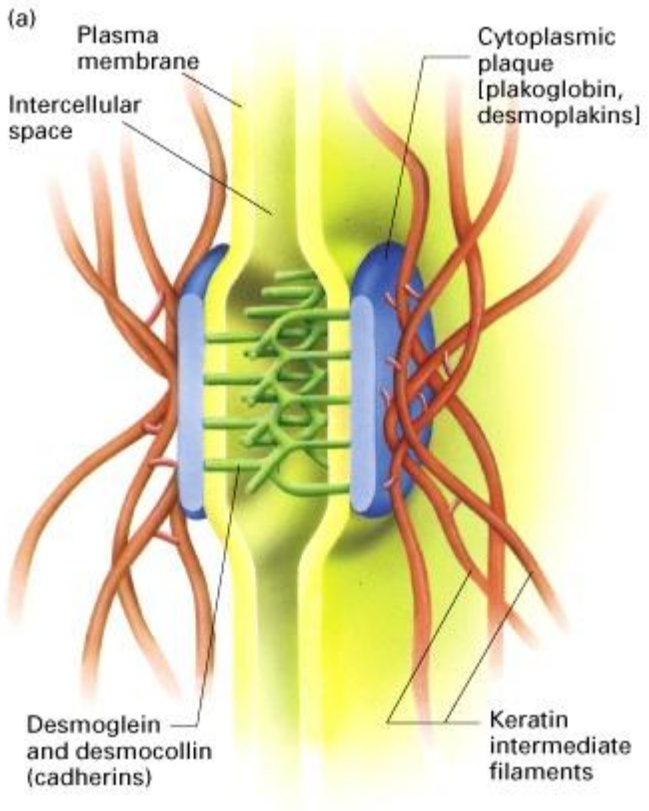
1. ZONULAE ADHERENS

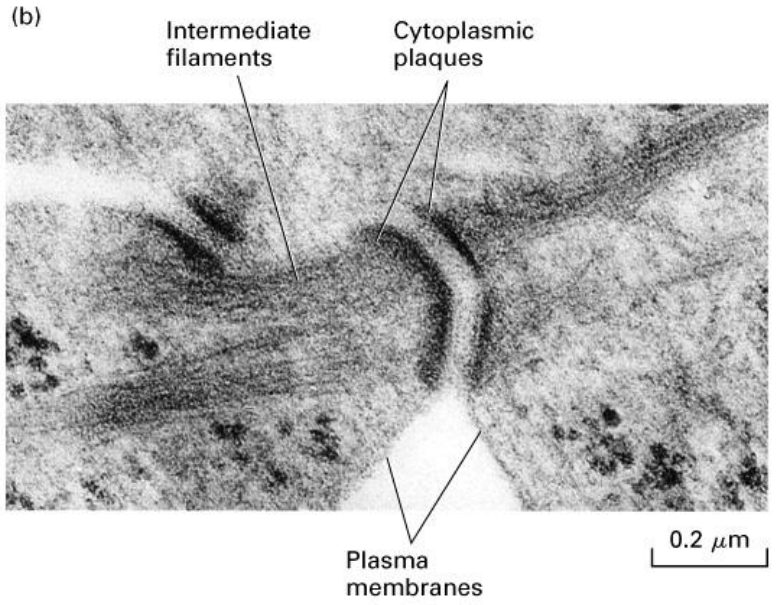
Banda continua di molecole di caderina che unisce le membrane laterali delle cellule epiteliali. Questa regione contiene le α- e β- catenine, che associano le molecole di E-caderina presenti sulla membrana plasmatica ad un fascio anulare intercellulare di actina e miosina. Nelle ***giunzioni aderenti*** sono presenti molte delle proteine che mediano le adesioni focali, comprese la vinculina, la tropomiosina e l' α-actina. In associazione con la ***zonula adherens***, il fascio anulare intercellulare funziona come una fune portante che sostiene dall'interno la cellula e che, di conseguenza ne controlla la forma



2. *DESMOSOMI*

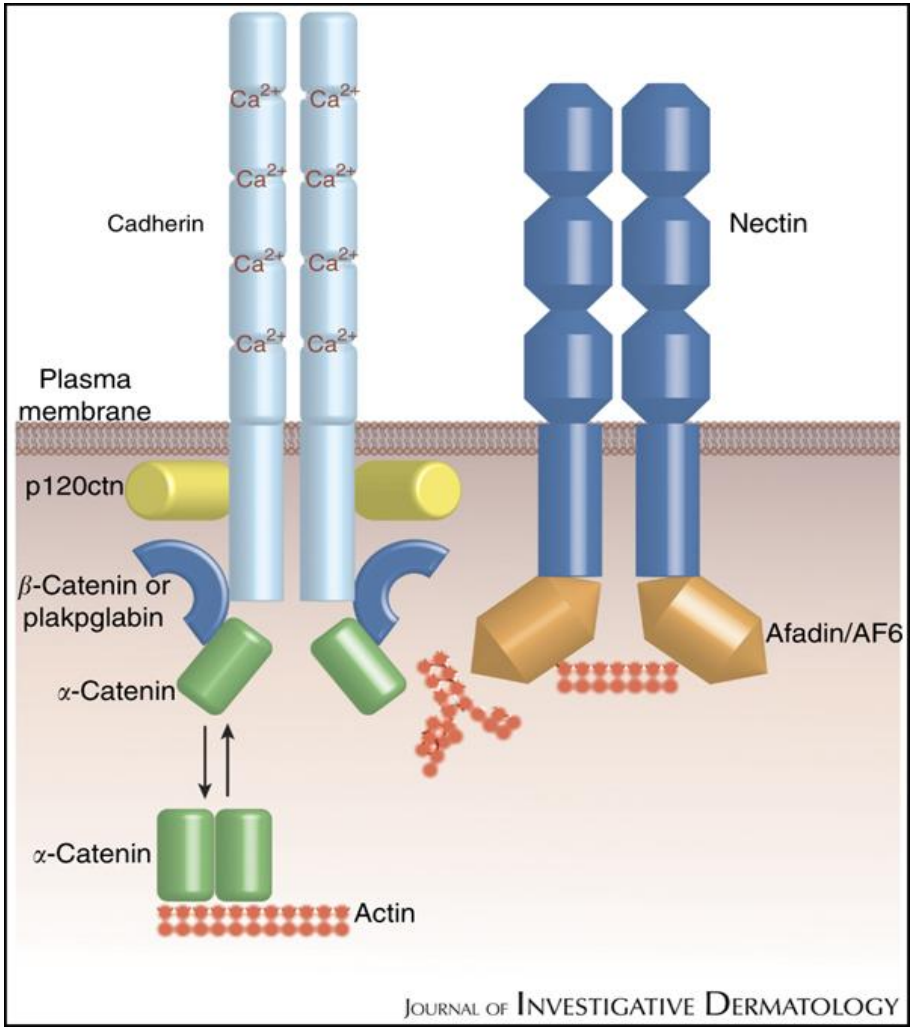
Un desmosoma e' formato da placche proteiche di adesione attaccate alla superficie citosolica delle membrane plasmatiche di cellule adiacenti e collegate da proteine transmembrana di connessione. Le placche sono costituite soprattutto da ***placcoglobina***, una proteina molto simile alla β- catenina. Le proteine transmembrana di connessione, chiamate ***desmogleina*** e ***desmocollina***, appartengono alla famiglia delle caderine. Si legano alla placcoglobina ed ad altre proteine delle placche e si estendono nello spazio intercellulare, dove interagiscono tra loro formando una rete che unisce due cellule assieme.





Proteine di connessione

caderine – citoscheletro

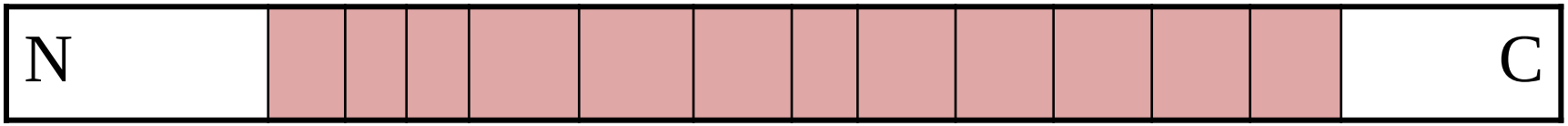


Caderina: dominio intracellulare

- 150aa, dominio più conservato
- se non legato, non è strutturato
- mentre viene sintetizzato nel RE lega la β catenina
- le due proteine sono trasportate insieme alla superficie cell
- contiene seq PEST (ubiquitinazione), di solito schermata dalla β catenina (regolazione)

β catenina

770-780aa



150aa

520aa
12 ripetizioni “arm”

100aa

Dominio arm
- H1
- H2
- H3

α Catenina
Siti di fosforilazione

Caderina
(o TCF)

coattivatori

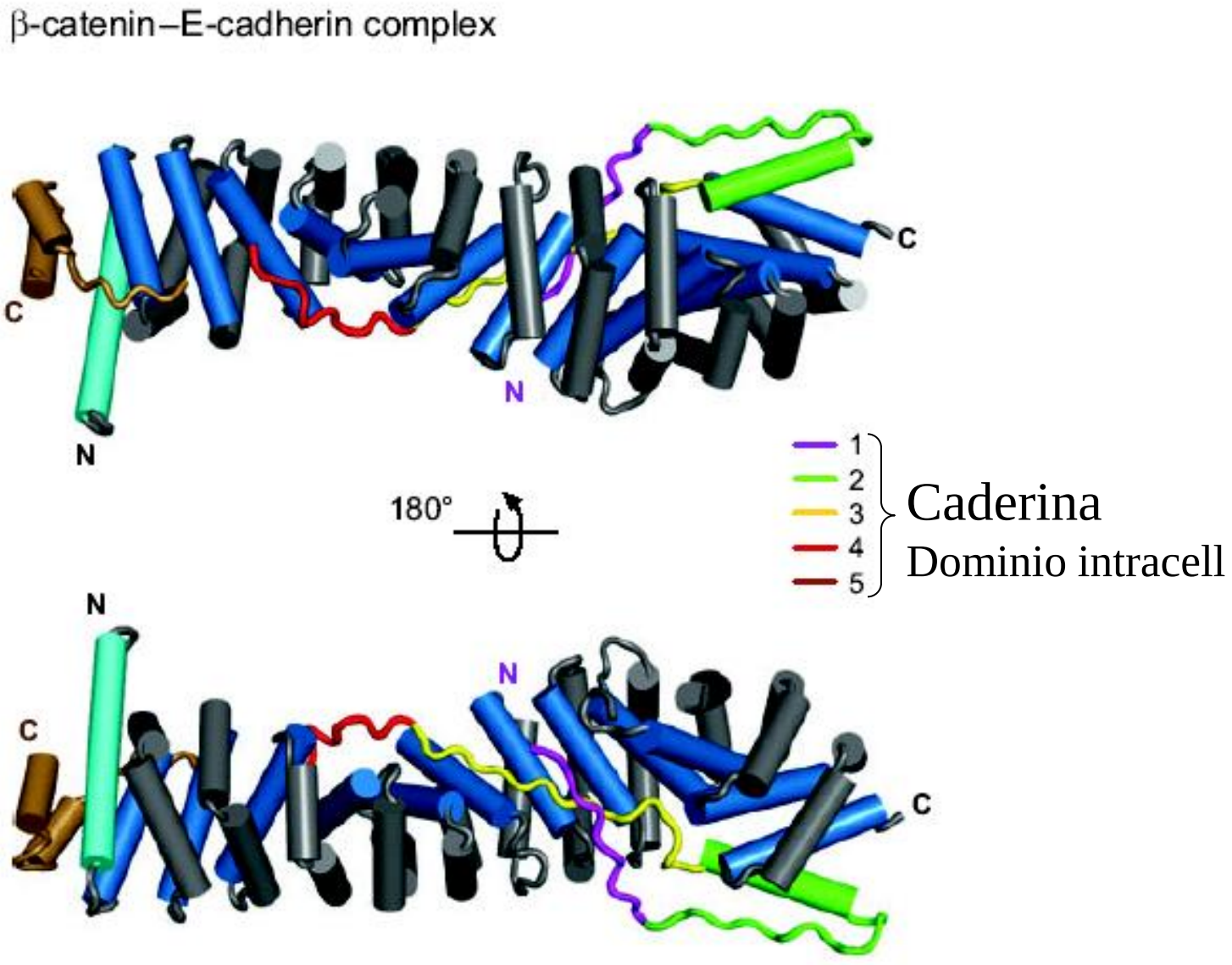
Arm:

H1

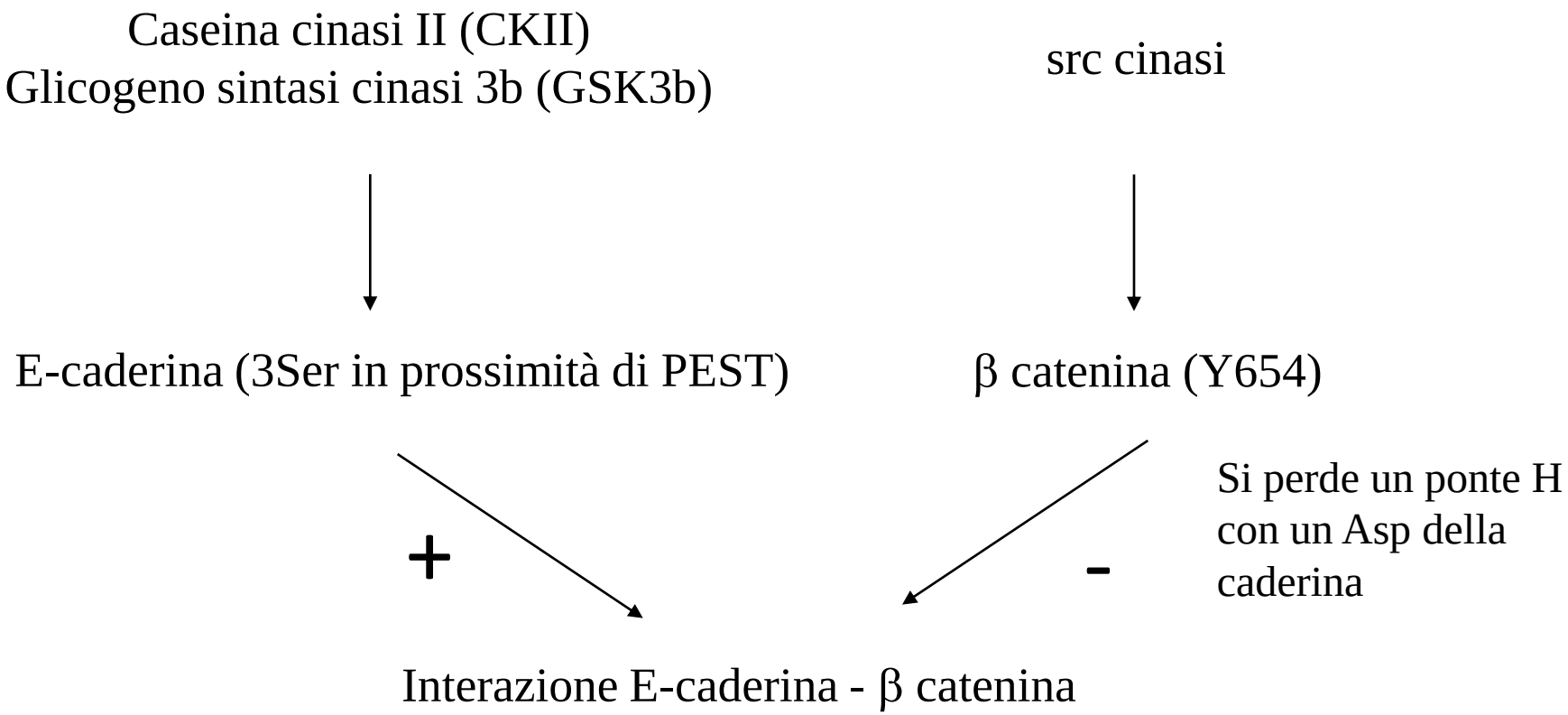
H2

H3

superelica



L’interazione caderina - β catenina è modulata da fosforilazioni:



β catenina

•Lega fattori trascrizione (Wnt signalling): coattivatore trascrizionale

•Lega APC/axina: degradazione

•Può essere fosforilata da Abl (Y489), si stacca da caderina e raggiunge il nucleo

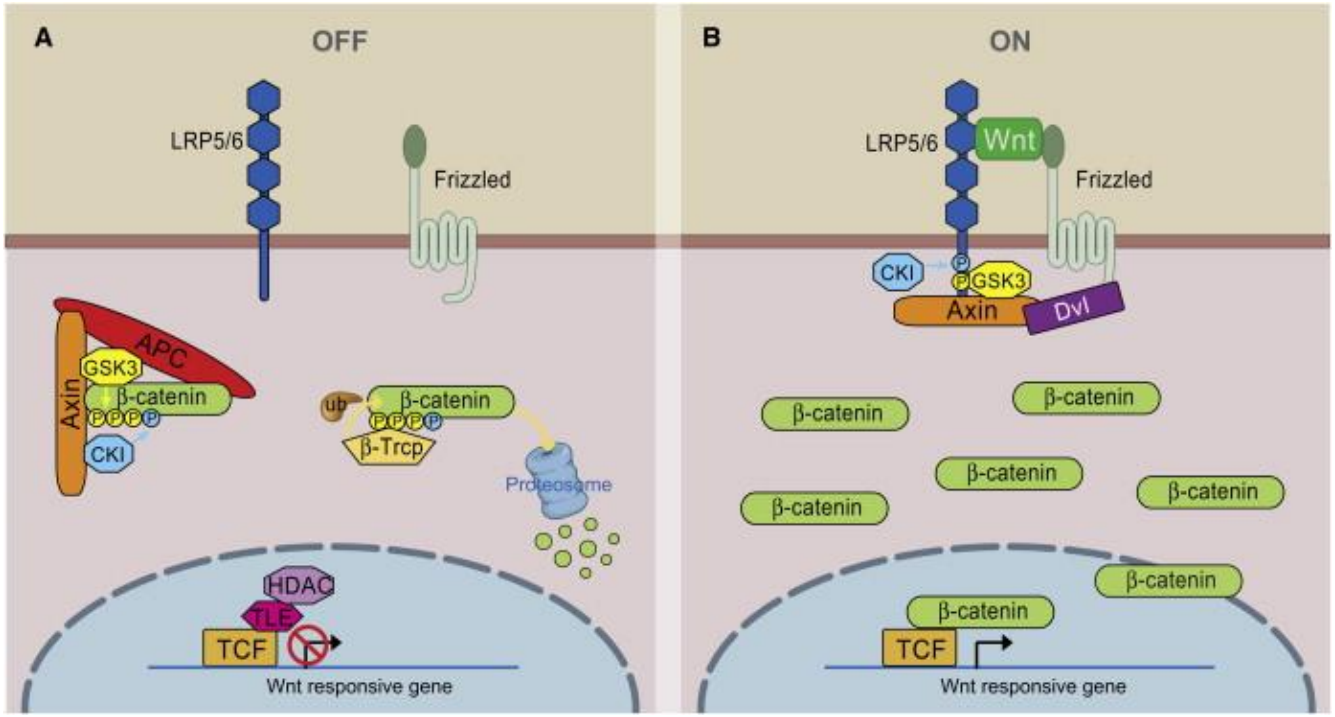


Figure 1. Overview of Wnt/β-catenin signaling

A) In the absence of Wnt, cytoplasmic β-catenin forms a complex with Axin, APC, GSK3 and CK1, and is phosphorylated by CK1 (blue) and subsequently by GSK3 (yellow). Phosphorylated β-catenin is recognized by the E3 ubiquitin ligase β-Trcp, which targets β-catenin for proteosomal degradation. Wnt target genes are repressed by TCF-TLE/Groucho and histone deacetylases (HDAC). B) In the presence of Wnt ligand, a receptor complex forms between Fz and LRP5/6. Dvl recruitment by Fz leads to LRP5/6 phosphorylation, and Axin recruitment. This disrupts Axin-mediated phosphorylation/degradation of β-catenin, allowing β-catenin to accumulate in the nucleus where it serves as a co-activator for TCF to activate Wnt responsive genes.

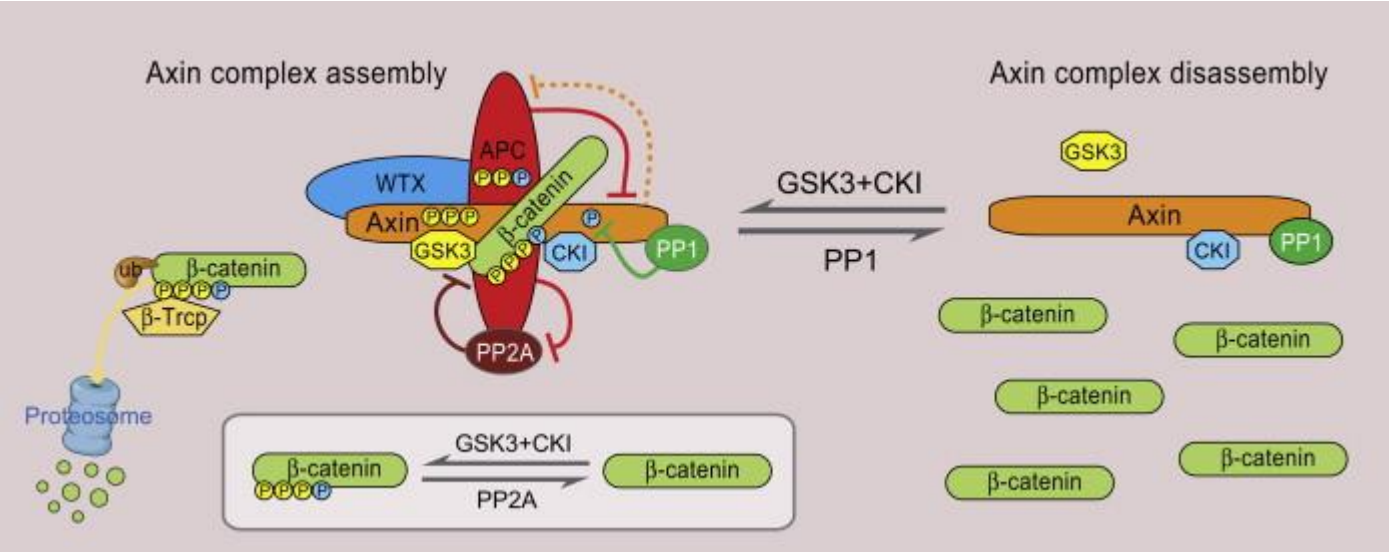


Figure 4. Regulation of Axin complex assembly for β-catenin degradation
The core components of the Axin complex, Axin, APC, GSK3 and CK1 collectively promote β-catenin phosphorylation for degradation by β-Trcp. In addition to phosphorylating β-catenin, GSK3 (yellow) and CK1 (blue) also phosphorylate Axin and APC and enhance their binding to β-catenin and degradation complex stability, further ensuring β-catenin phosphorylation. The inset illustrates β-catenin phosphorylation (by CK1 and GSK3) and dephosphorylation (by PP2A). APC may also act to prevent PP2A dephosphorylation of β-catenin. APC paradoxically facilitates Axin degradation and possibly vise vesa (indicated by dashed line, see text). PP1 dephosphorylates Axin to antagonize CK1 phosphorylation and negatively regulates GSK3-Axin binding resulting in complex disassembly.

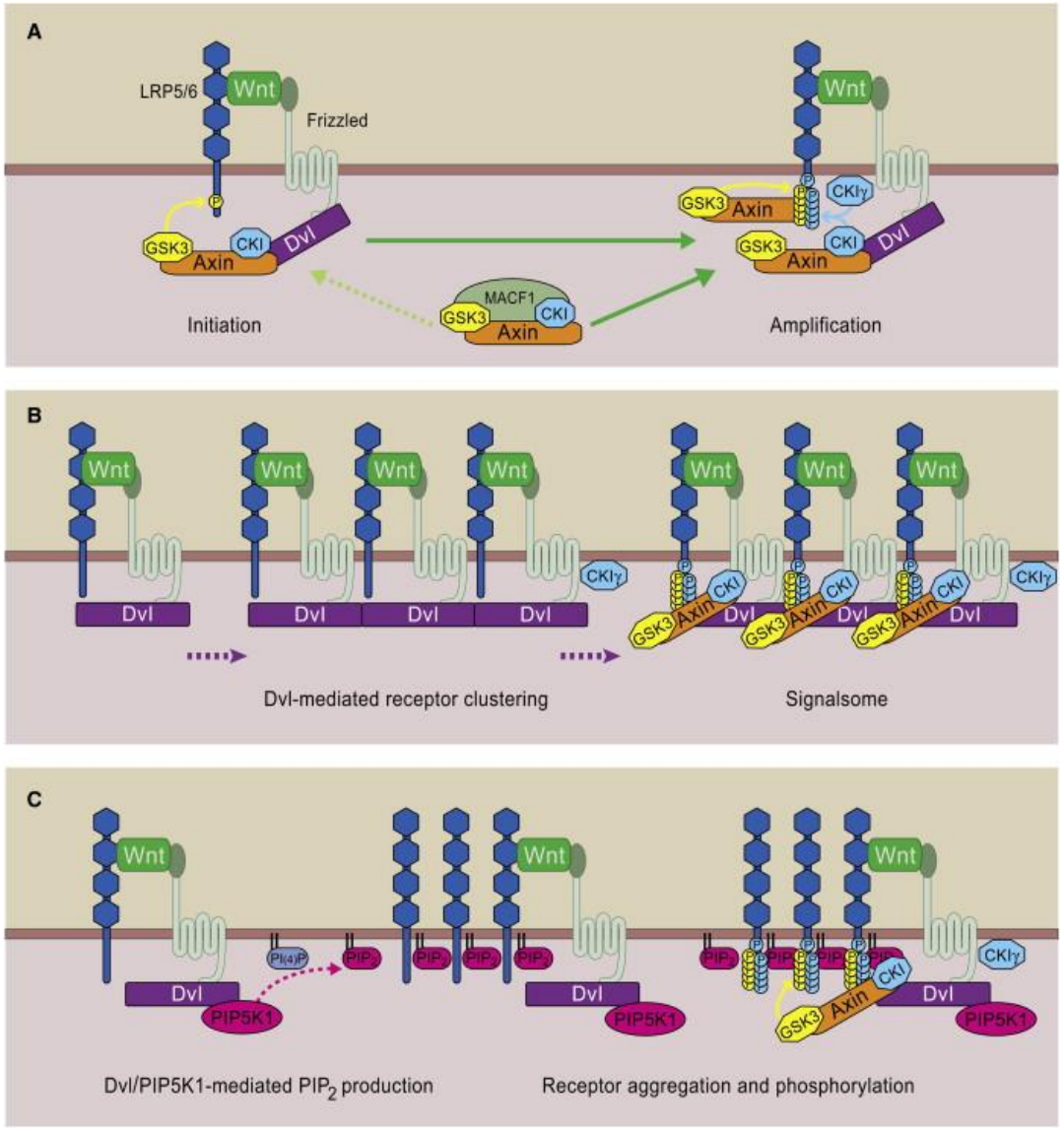
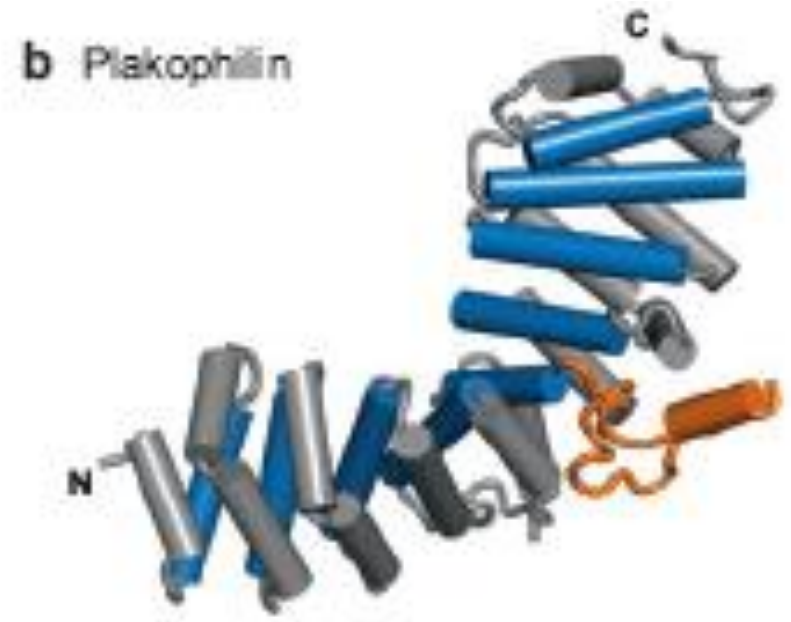
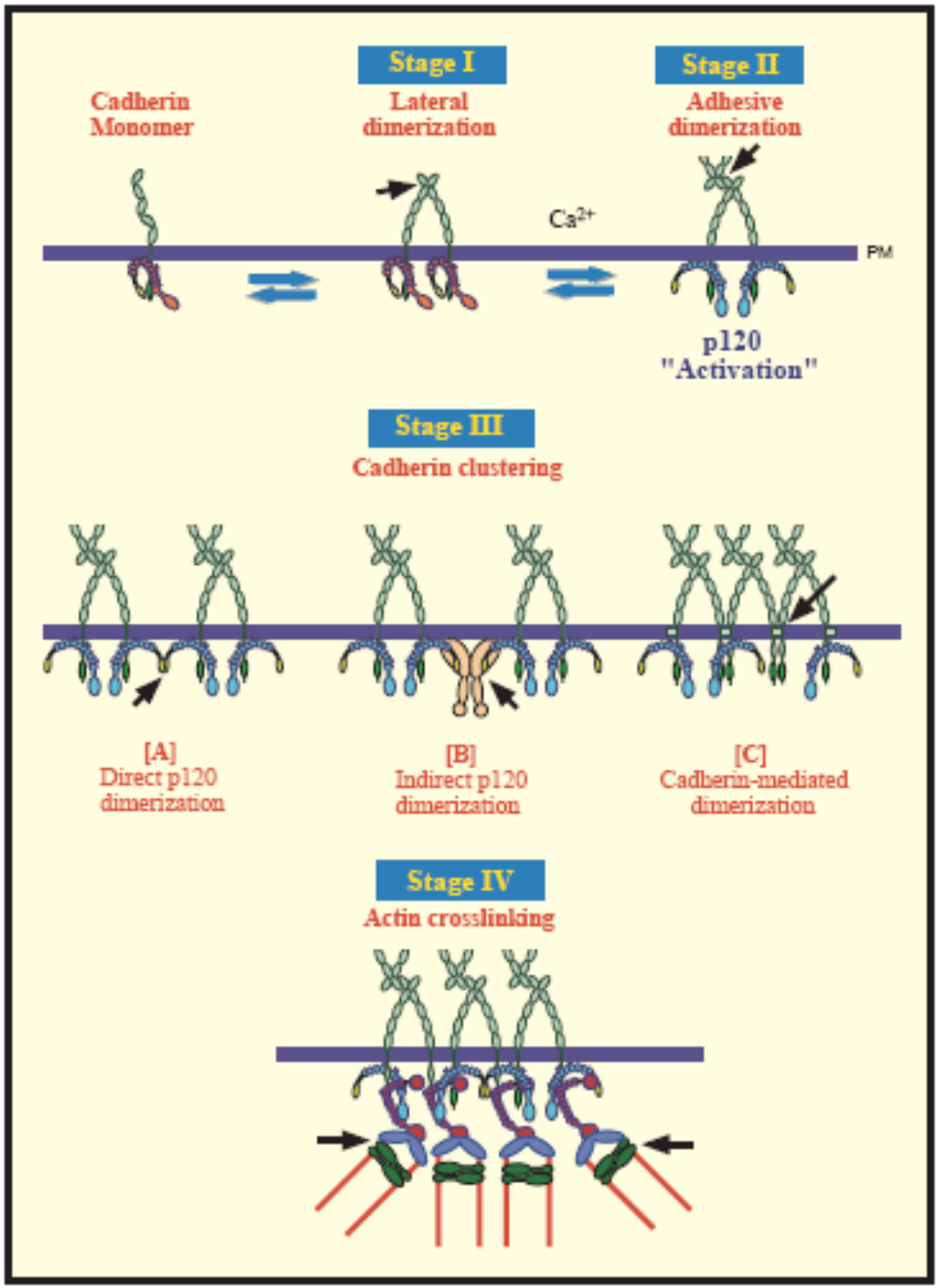


Figure 5. Models of Wnt receptor activation
A) Initiation and Amplification. Wnt forms a complex with LRP6 and Fz-Dvl at the membrane. Dvl recruits Axin-GSK3 resulting in the phosphorylation of one or more PPPSP motifs in LRP6 (initiation). Partially phosphorylated LRP6 may be able to recruit and more efficiently bind Axin-GSK3 and promote more PPPSP phosphorylation (amplification). B) Signalsome formation via Dvl polymerization and receptor clustering. The oligiomerization property of Dvl promotes the aggregation of individual Wnt-LRP6-Fz complexes, resulting in Axin recruitment to the membrane and LRP6 phosphorylation by GSK3 and CK1. C) PI4KIIα and PIP5KI kinases, the latter of which binds directly with Dvl, promote PIP2 production and receptor clustering/phosphorylation. The configurations of receptor clustering in B and C were drawn arbitrarily. In all models, PPPSPxS motifs are sequentially phosphorylated by GSK3 and CK1, probably via CK1γ (membrane-associated) and/or CK1α and CK1ε associated with Axin and Dvl, respectively, and MACF1 may have a role in the translocation of the Axin complex to the receptors.

p120

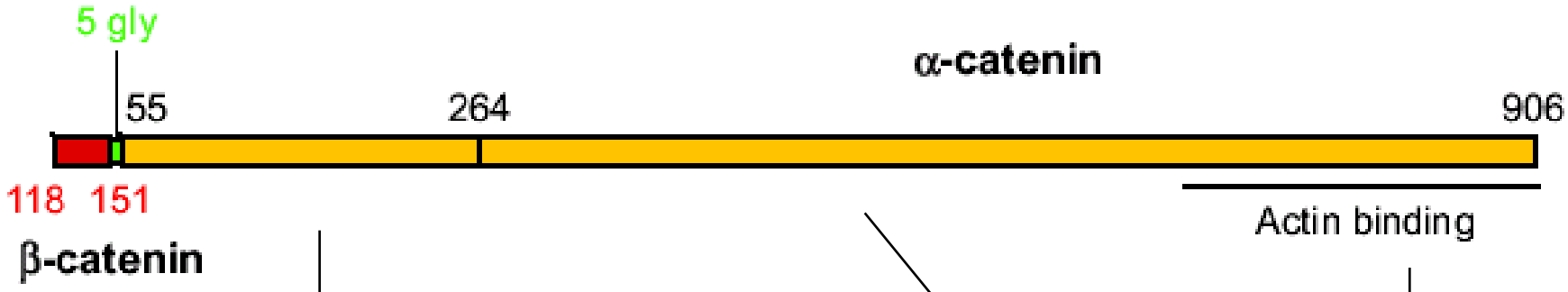


- 9 ripetizioni ARM
- Lega regione justamembrana delle caderine
- Regola internalizzazione/degradazione caderine
- Regola interazioni *cis* tra caderine


Stage I
Lateral dimerization
Stage II
Adhesive dimerization
Cadherin Monomer
Ca2+
PM
p120 "Activation"
Stage III
Cadherin clustering
[A] Direct p120 dimerization
[B] Indirect p120 dimerization
[C] Cadherin-mediated dimerization
Stage IV
Actin crosslinking

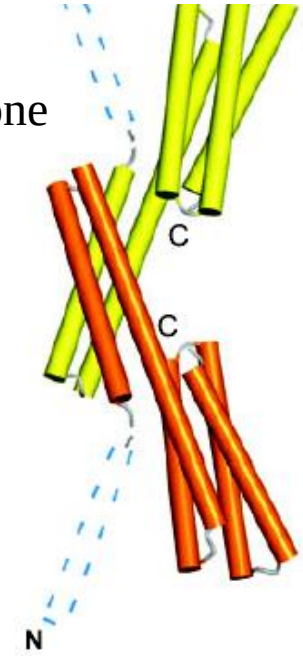
α catenina

906aa



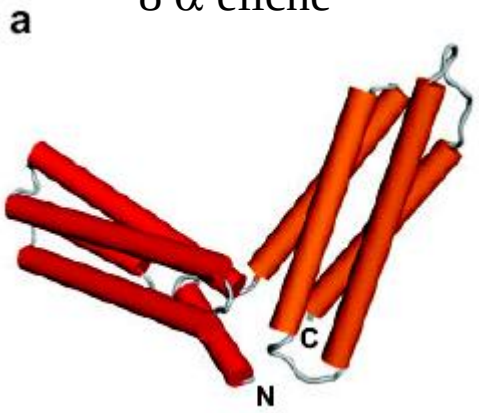
Dominio di dimerizzazione

5 α eliche

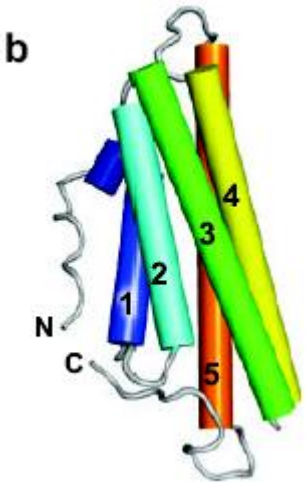


Dominio M

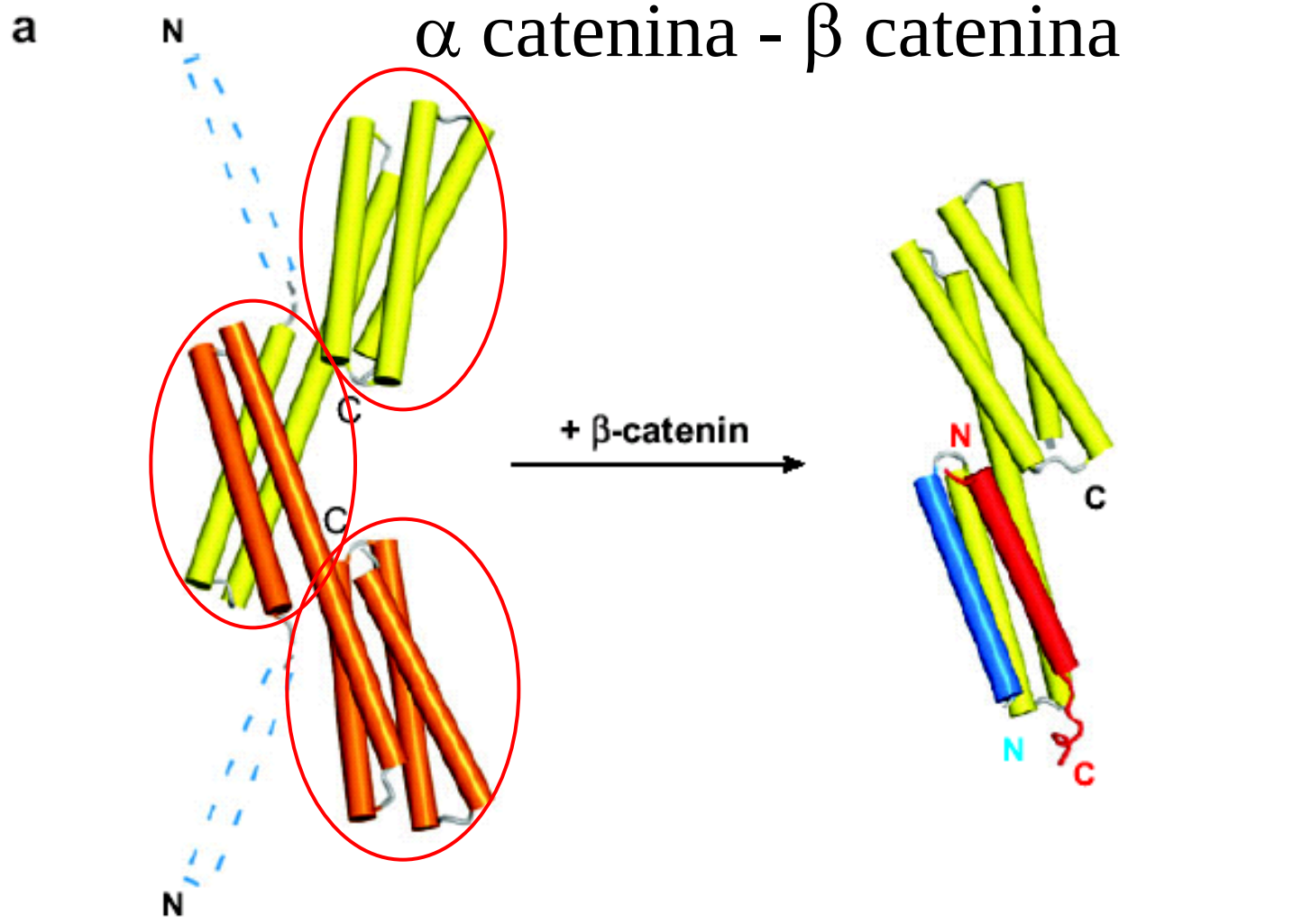
8 α eliche



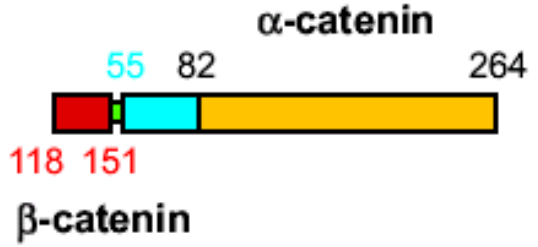
5 α eliche
simile vinculina



α catenina - β catenina



Fasci di 4 eliche antiparallele



b

α catenina

Complesso caderina - β catenina – α catenina – actina?

α Catenina monomerica o dimerica

Regolazione polimerizzazione actina e formazione giunzioni aderenti?

Lamellipodi (altamente dinamici, actina puntiforme)

↓

Membrana più uniforme, formazione di fibre da stress

Figure 7

Model of α-catenin function in actin polymerization and reorganization in developing adherens junctions. Initial contacts between cadherins on opposed lamellipodia lead to cadherin clustering. α-Catenin bound to the clustered E-cadherin–β-catenin complex rapidly exchanges between β-catenin and the cytosol, thereby producing a locally high concentration of α-catenin that can dimerize and suppress the activity of Arp2/3.

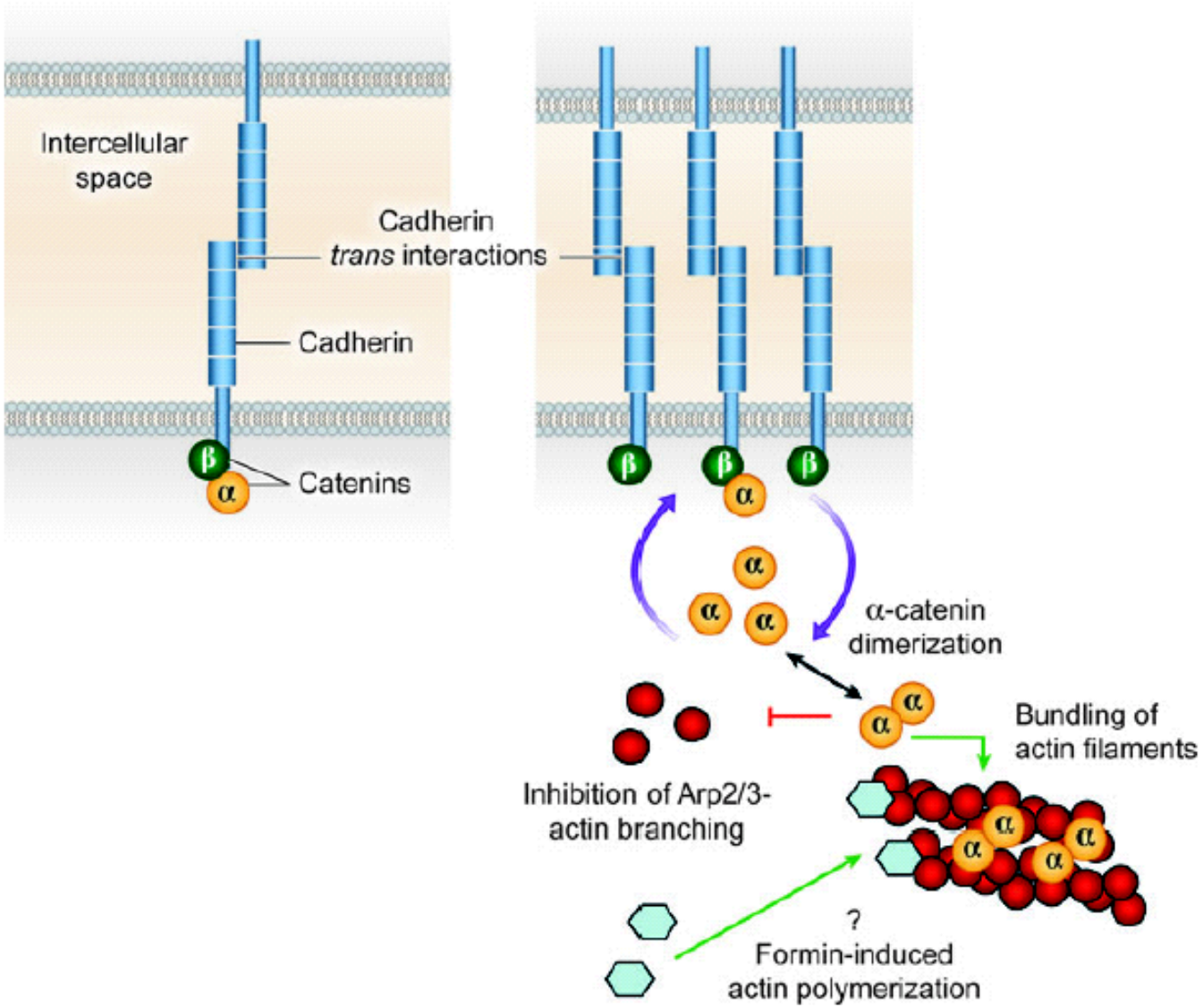
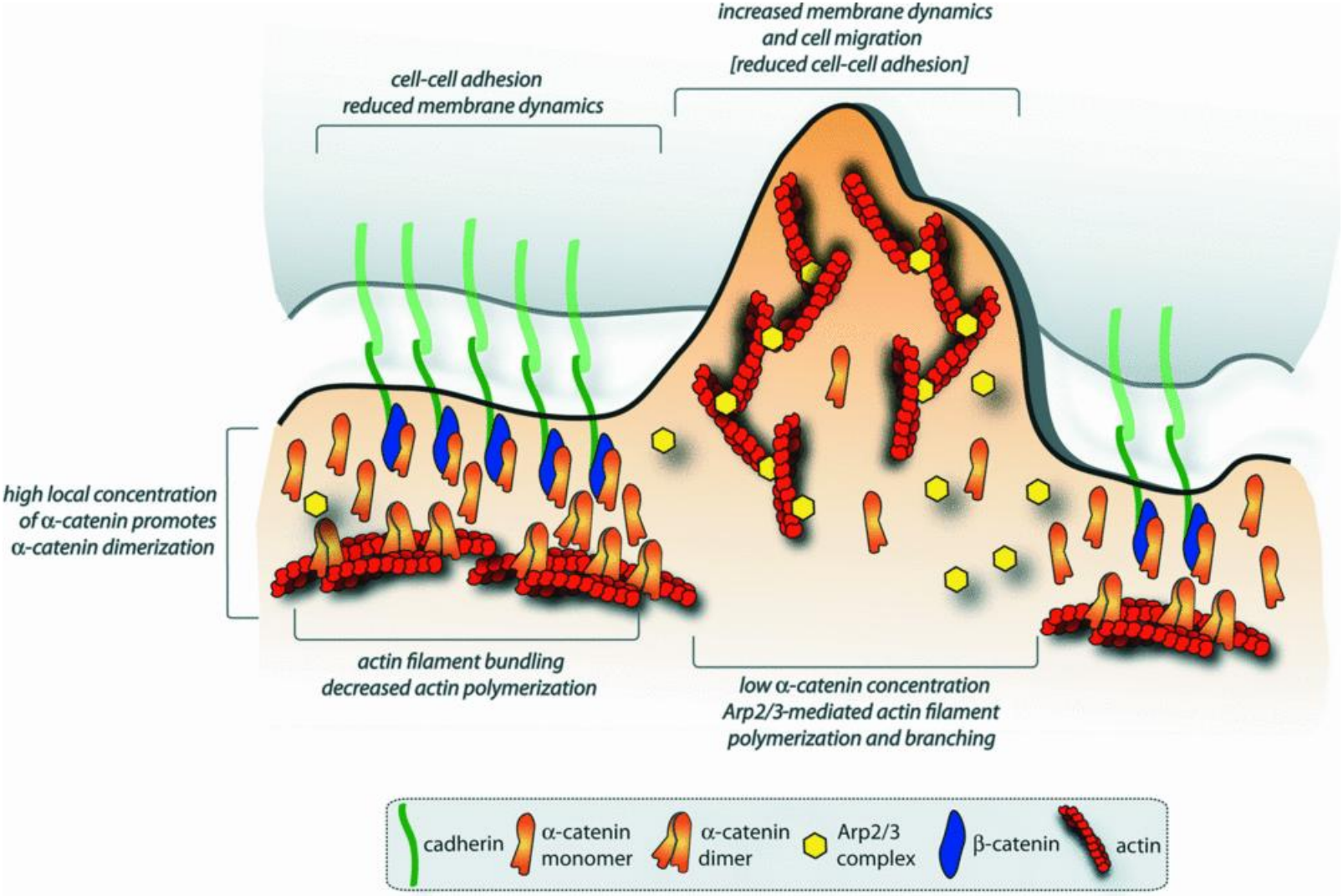
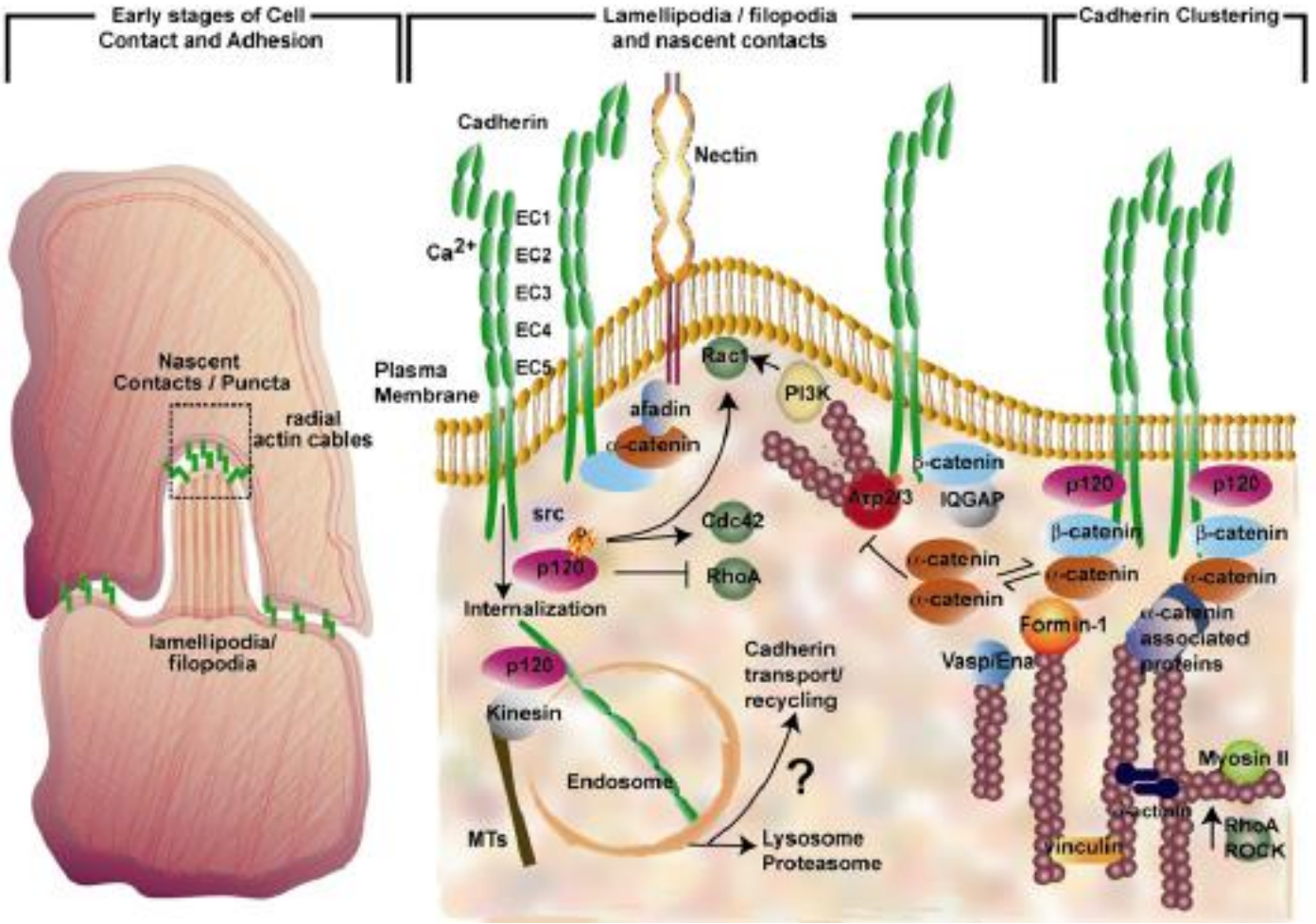


Figure 2 A model for regulation of cytoskeleton and membrane dynamics by the cadherin-catenin complex



Nelson W.J., Biochem. Soc. Trans. (2008) 36, 149-155



During the initial stages of intercellular adhesion, cells extend filopodial/lamellipodial extensions that enhance cell-cell contacts. Such dynamic membrane protrusive activity involves the actin cytoskeleton and Rho GTPases. Cadherin-catenin complexes are recruited to these nascent contacts. These events are orchestrated by both α-catenin and p120-catenin. When more nascent contacts begin to form, the densities of AJ-associated proteins, including α–catenin, rise and promote the formation of α-catenin homodimers that may then serve as a feedback mechanism to dampen lamellipodial movements and promote the formation of radial actin cables as cell-cell junction formation progresses. At intermediate stages of intercellular adhesion, the lateral clustering of cadherins promotes the association of actin-binding and actin-polymerizing proteins. Under these conditions, membrane sealing is enhanced, and eventually radial actin cables also rearrange and get stabilized and bundled by myosin II, a-actinin, and possibly α-catenin homodimers. Establishment of mature cell contacts reorganizes the actin cytoskeleton to this more static state.

Caderine

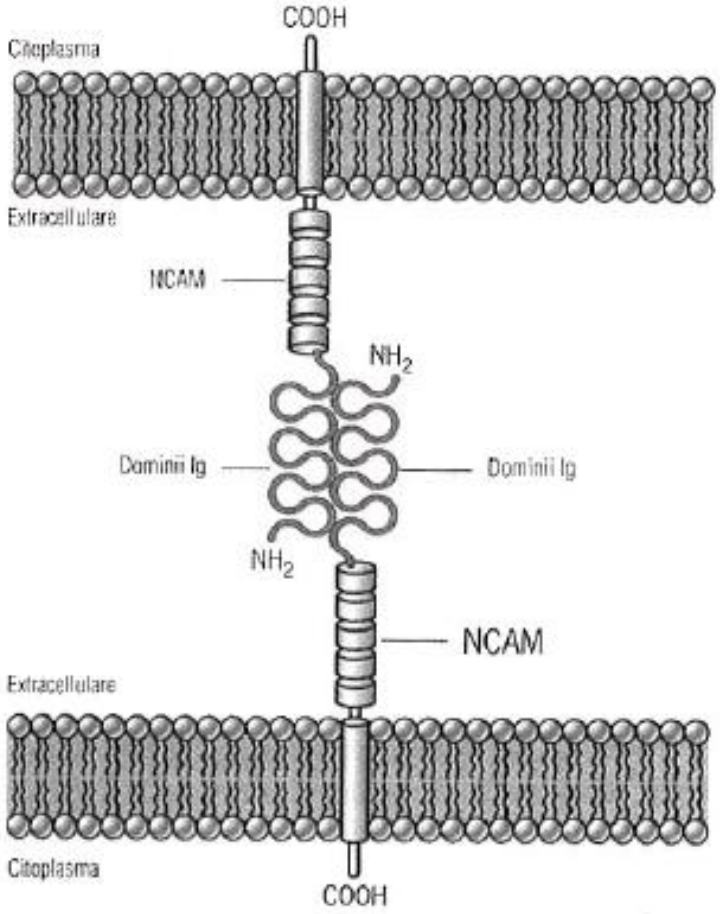
-Ruolo strutturale (actina)

-Ruolo di segnalazione:

Sequestro β-catenina (inibizione da contatto)

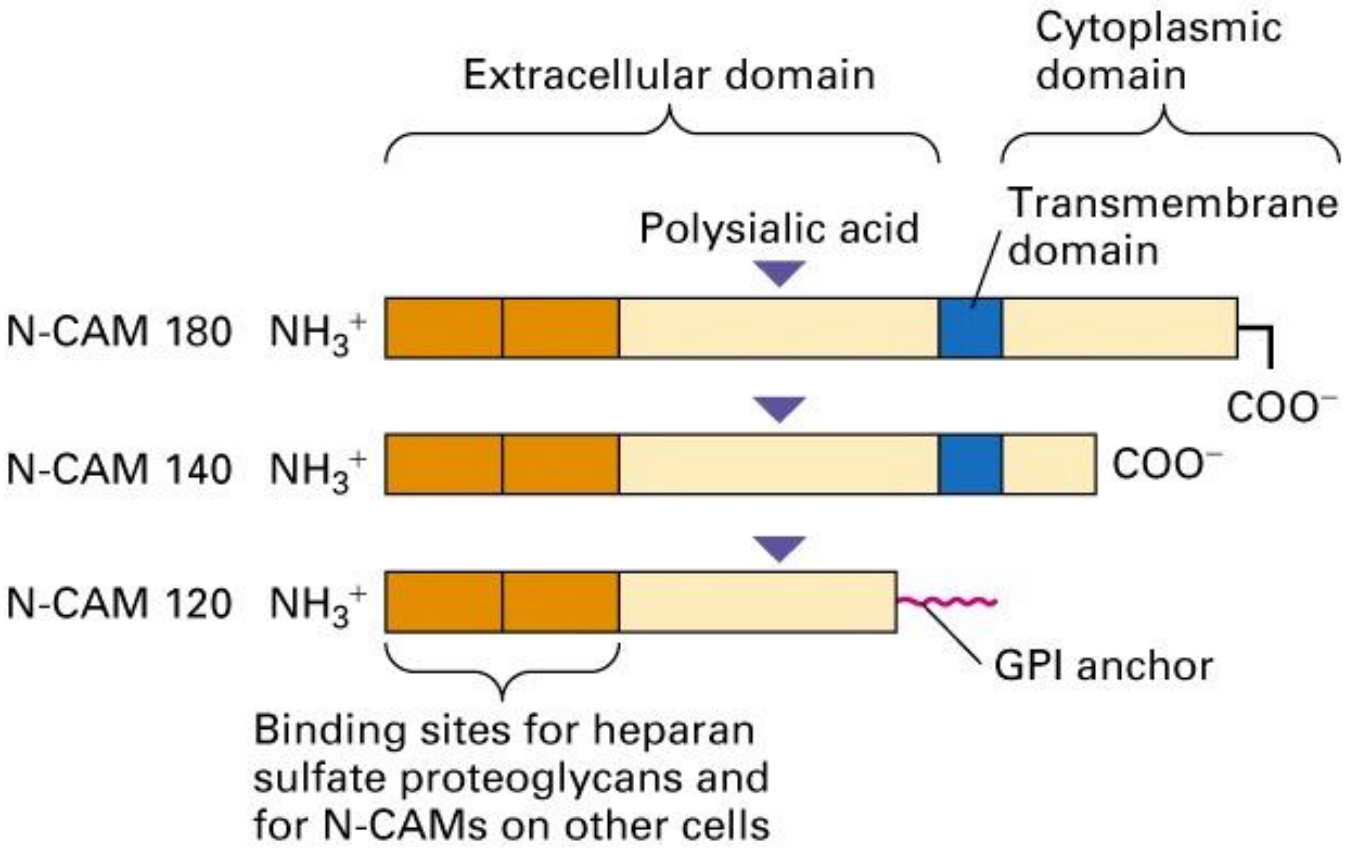
Attivazione enzimi (Rac-1)

CAM della superfamiglia delle immunoglobuline: N-CAM

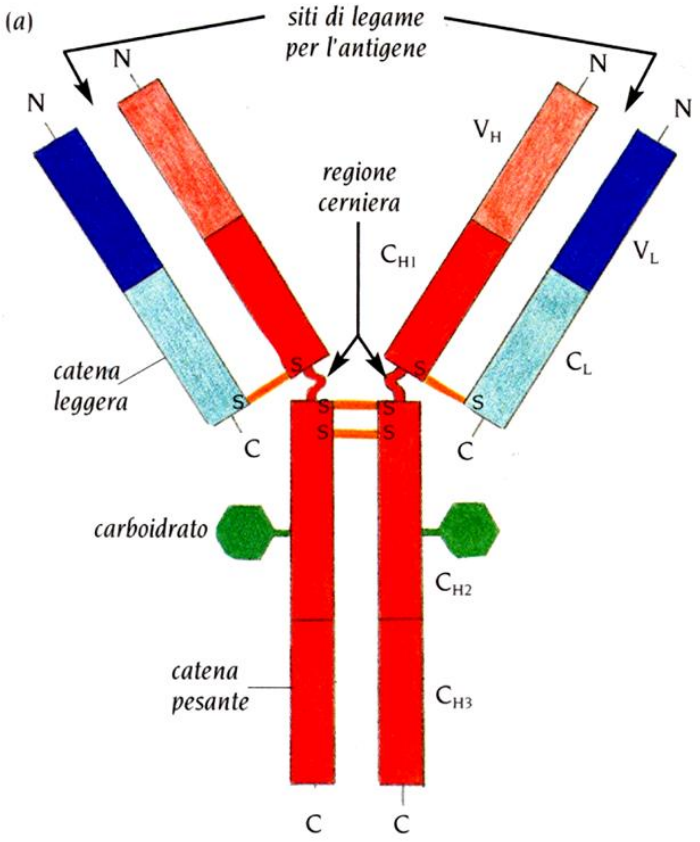


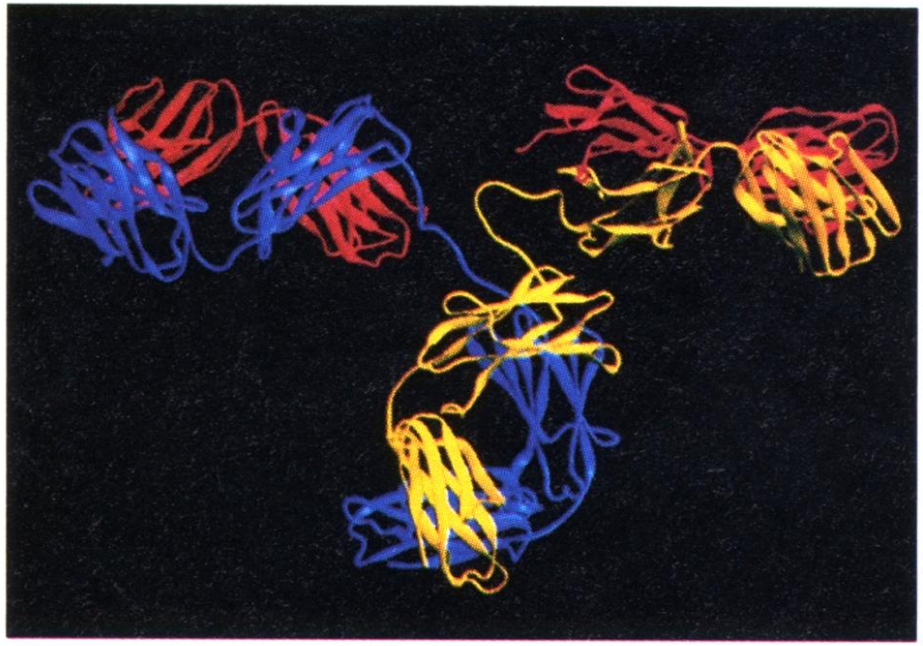
La superfamiglia delle immunoglobuline comprende gli anticorpi e un'ampia varietà di proteine transmembrana;
alcune di queste ultime mediano l'adesione cellula-cellula **indipendente da ioni calcio.**
Strutturalmente queste proteine sono composte da una serie di domini simili. Ciascun dominio è composto da 70-120 amminoacidi organizzati in una struttura altamente ripiegata.

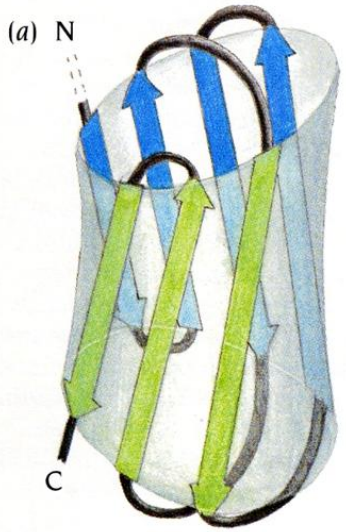
N-CAM

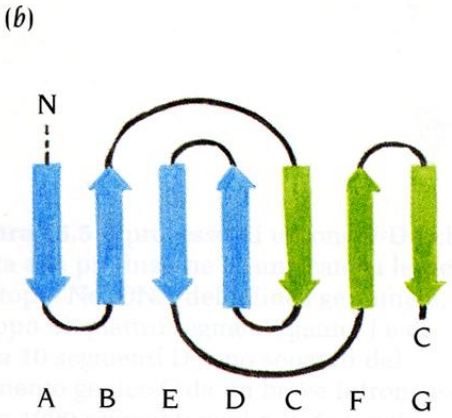

Extracellular domain
Cytoplasmic domain
Polysialic acid
Transmembrane domain
N-CAM 180
NH_3^+
COO^-
N-CAM 140
NH_3^+
COO^-
N-CAM 120
NH_3^+
GPI anchor
Binding sites for heparan sulfate proteoglycans and for N-CAMs on other cells

IgG


(a)
siti di legame per l'antigene
N
N
N
N
V_H
V_L
regione cerniera
C_{H1}
C_L
catena leggera
S
S
S
S
S
S
S
S
C
C
carboidrato
C_{H2}
catena pesante
C_{H3}
C
C

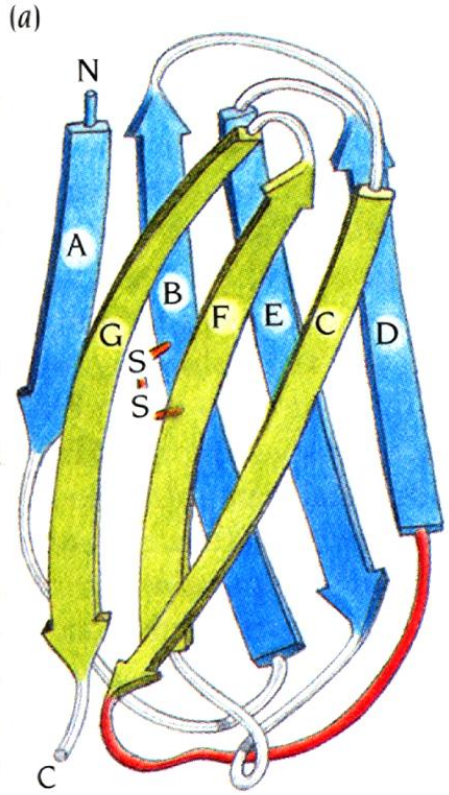




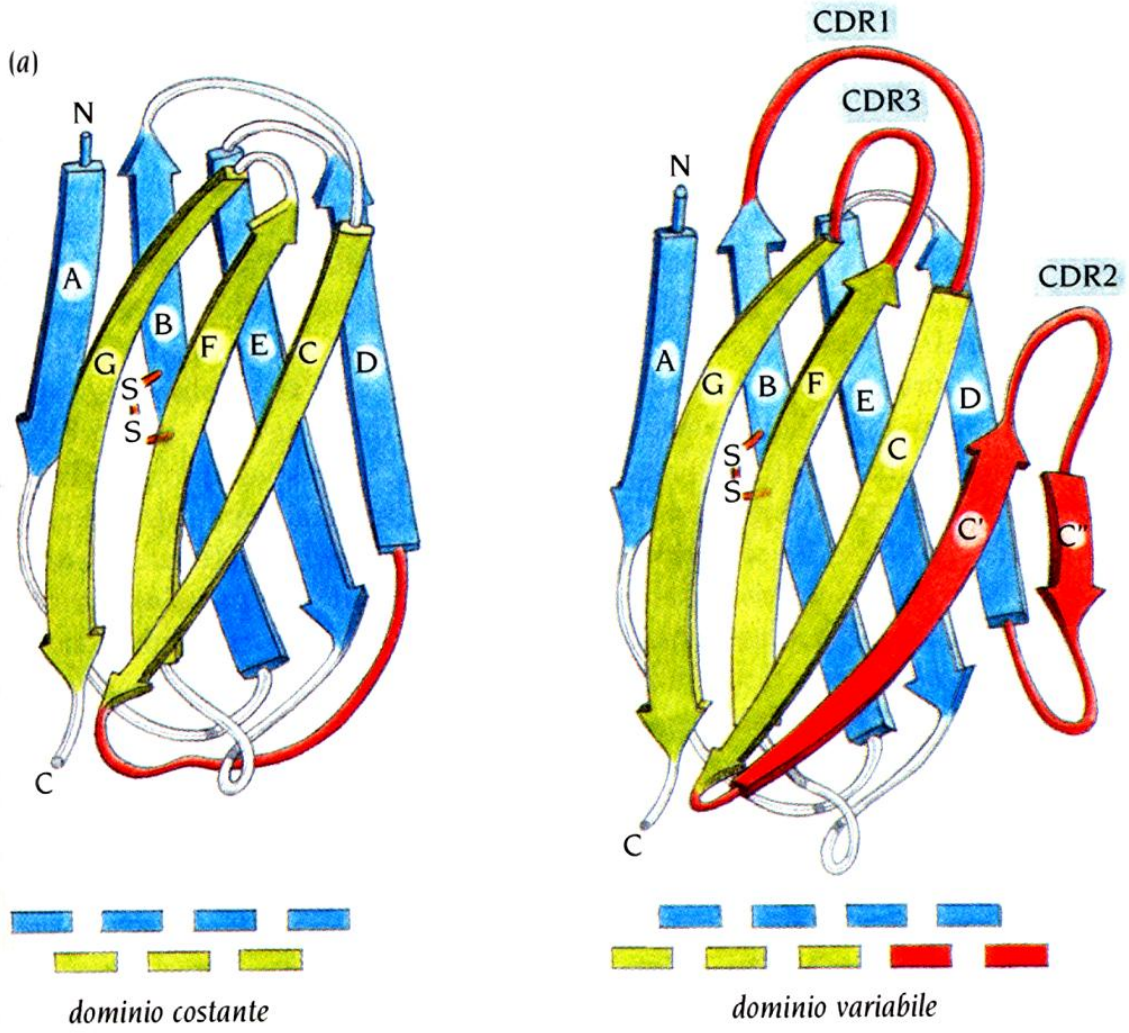


Ripiegamento Immunoglobulinico (chiave greca)

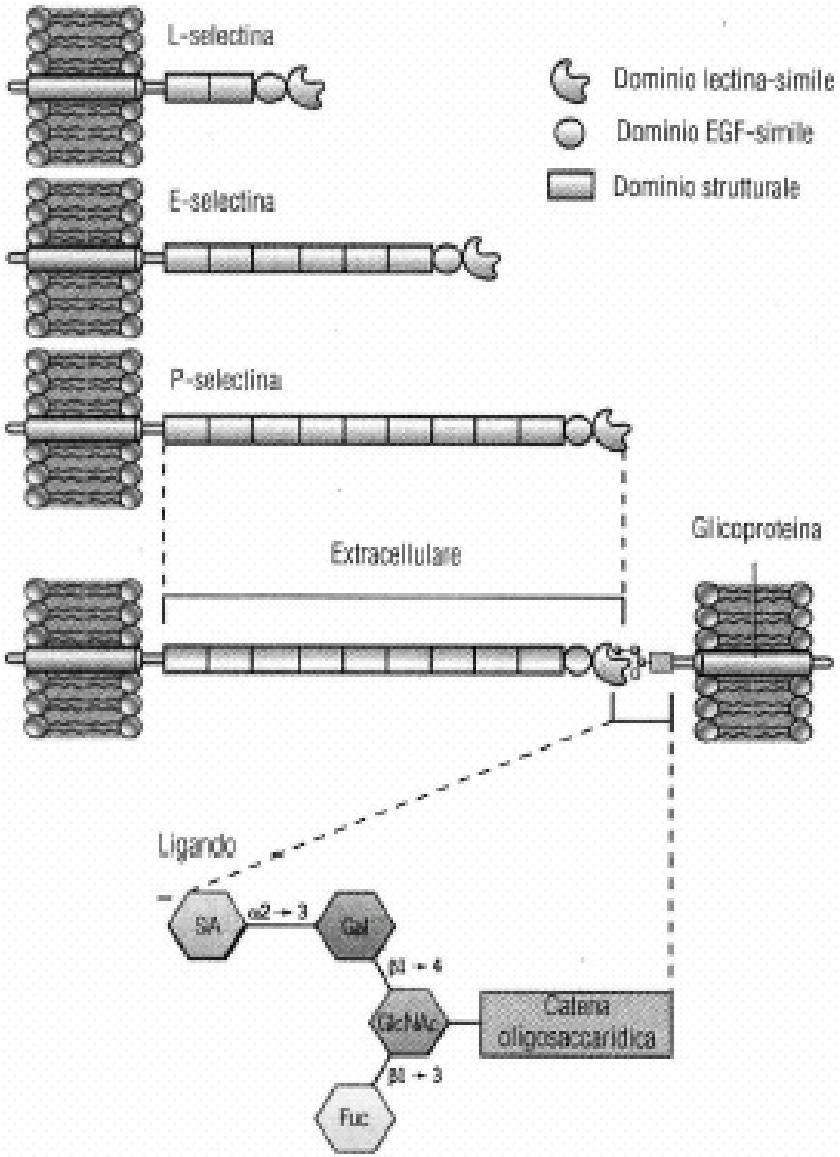
Dominio costante



A confronto con il dominio variabile

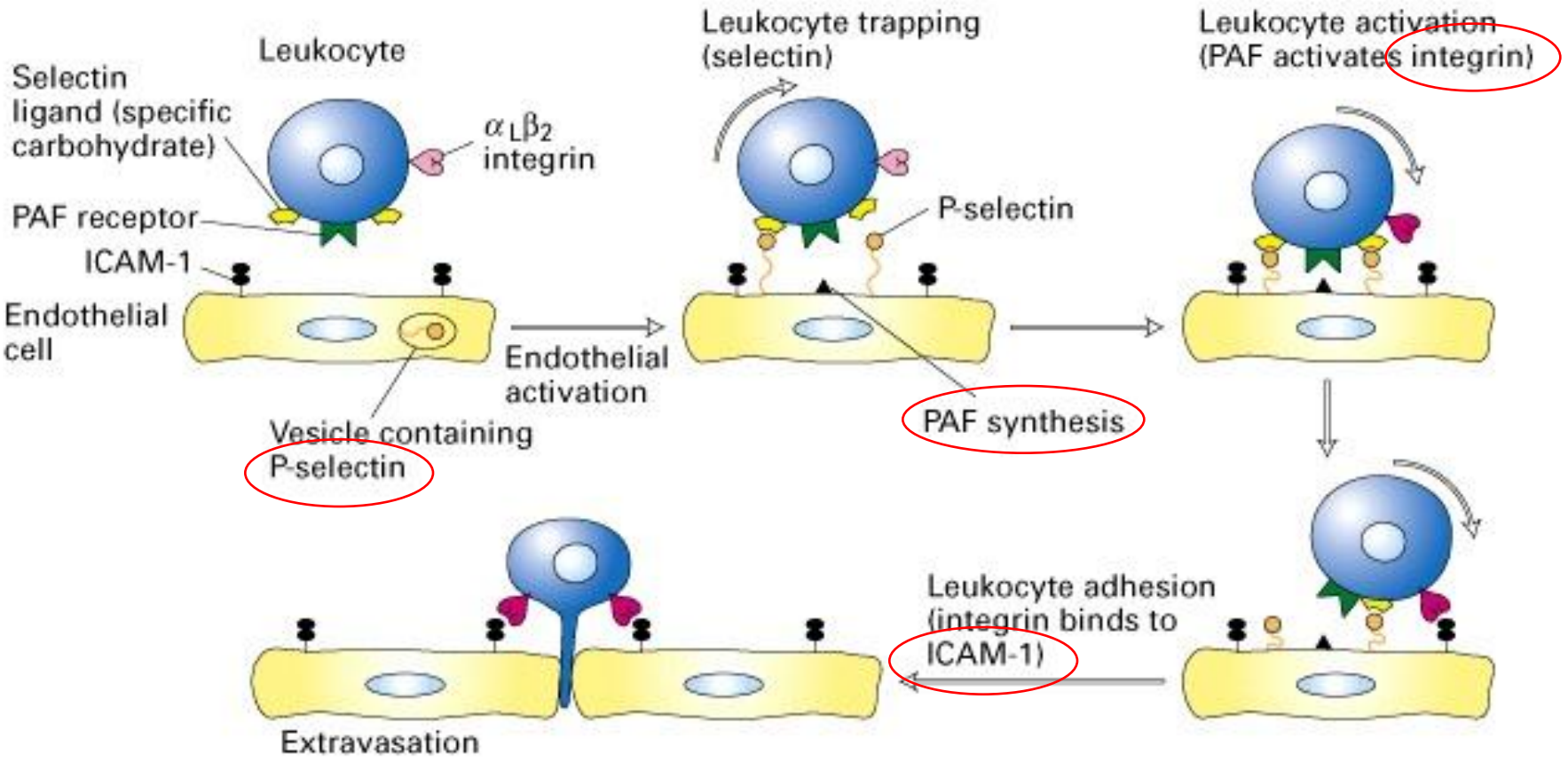


Selettine



Esistono tre tipi di selectine: L-selectina selectina, E-selectina e P-selectina.
Strutturalmente la proteina è composta da un dominio N-
terminale omologo alle **lectine** animali **calcio-dipendenti**, da un
dominio tipo EGF, da 2 a 9 domini seguiti da un tratto elicoidale transmembrana e da una corta coda citoplasmatica.
Le selectine mediano il processo di adesione cellulare tramite il riconoscimento, calcio dipendente, dei **glicani** sialiati (leg. eterofilico)
Sono importanti nelle interazioni transitorie cell-cell nel torrente circolatorio (rotolamento).

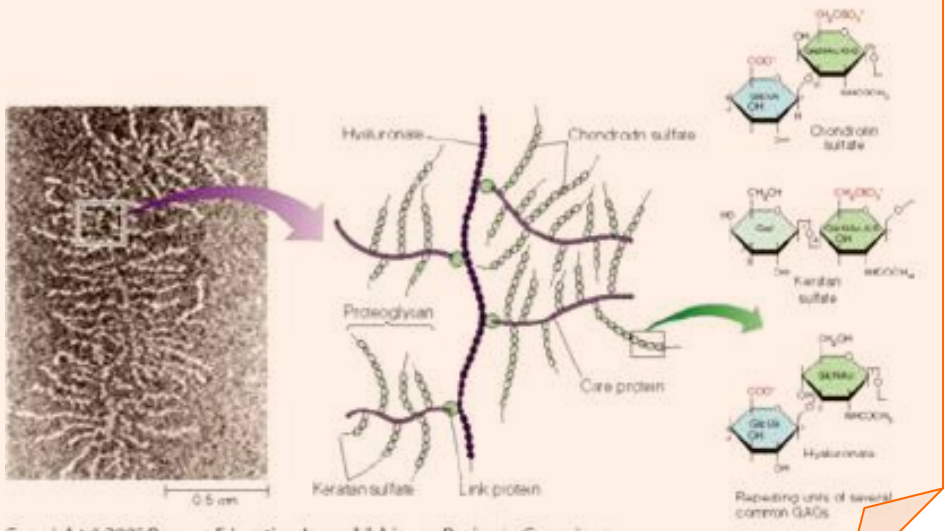
Selectins and other CAMs participate in leukocyte extravasation



ECM matrice extracellulare

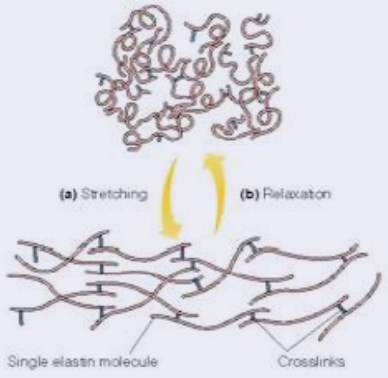
complessa rete di carboidrati e proteine secreti dalle cellule animali, ruolo strutturale e funzionale

Glicosamminoglicani (GAG) e Proteoglicani (PG)

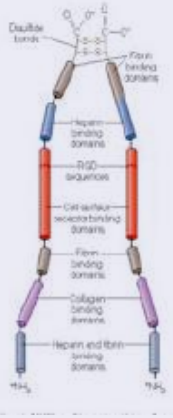


Proteine

1. *strutturali:* collagene-elastina…
2. *adesive: fibronettina, laminina, vitronettina….*



elastina



fibronettina

Collagene

- La più importante proteina fibrosa non solubile di ECM e del connettivo

- La più abbondante proteina nel regno animale

I tipi di collagene

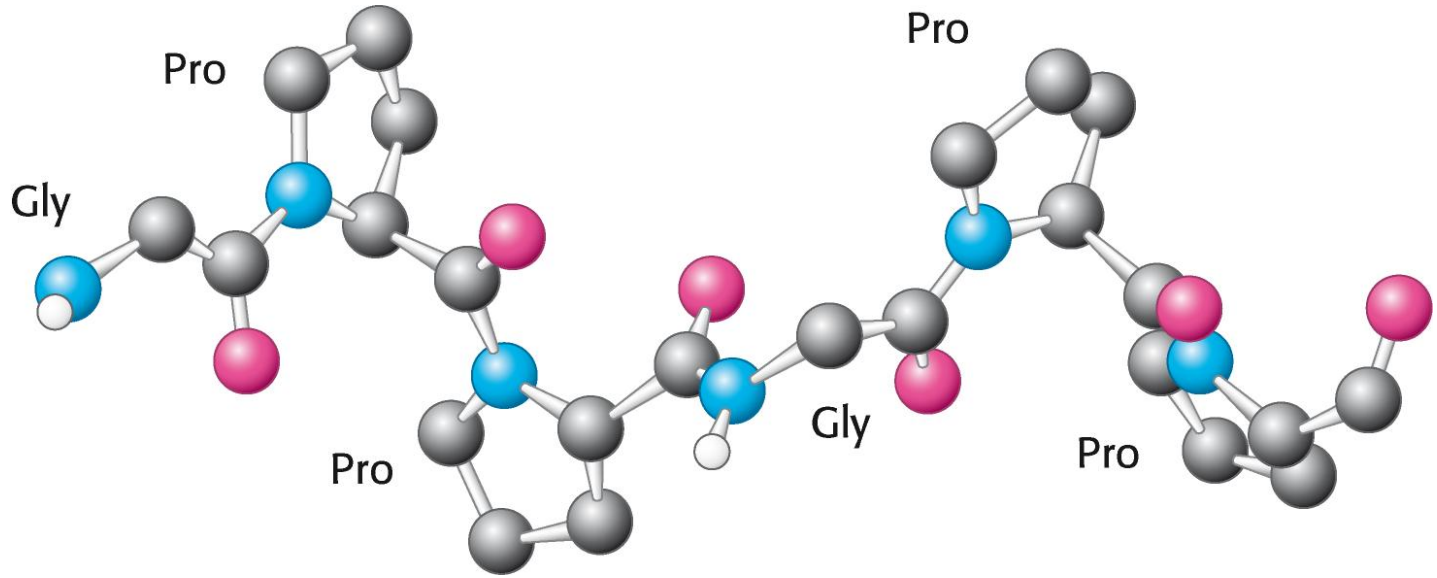
Tipo	*Catene*	*Struttura*	*Localizzazione*
I	α1(I), α2(I)	Fibrillare	Cute, tendini, osso, ecc.
II	α1(II)	Fibrillare	Cartilagine, corpo vitreo
III	α1(III)	Fibrillare	Cute, muscoli, ecc.
IV	α1(IV), α2(IV)	Non fibrillare	Tutte le membrane basali
V	α1(V), α2(V), α3(V)	Fibrillare	Molti tessuti interstiziali
VI	α1(VI), α2(VI), α3(VI)	Microfibrille	Molti tessuti interstiziali
VII	α1(VII)	Non fibrillare	Fibrille ancoranti
VIII	α1(VIII)	?	Alcune cellule endoteliali
IX	α1(IX), α2(IX), α3(IX)	?	Cartilagine
X	α1(X)	?	Cartilagine ipertrofica e mineralizzata
XI	α1(XI), α2(XI), α3(XI)	Fibrillare	Cartilagine
XII	α1(XII)	?	Cute, tendini

Da K. Kuhn, in: *Structure and Function of Collagen Types* (a cura di R. Mayne e R. E. Burgeson), Academic Press, 1987, p.2.

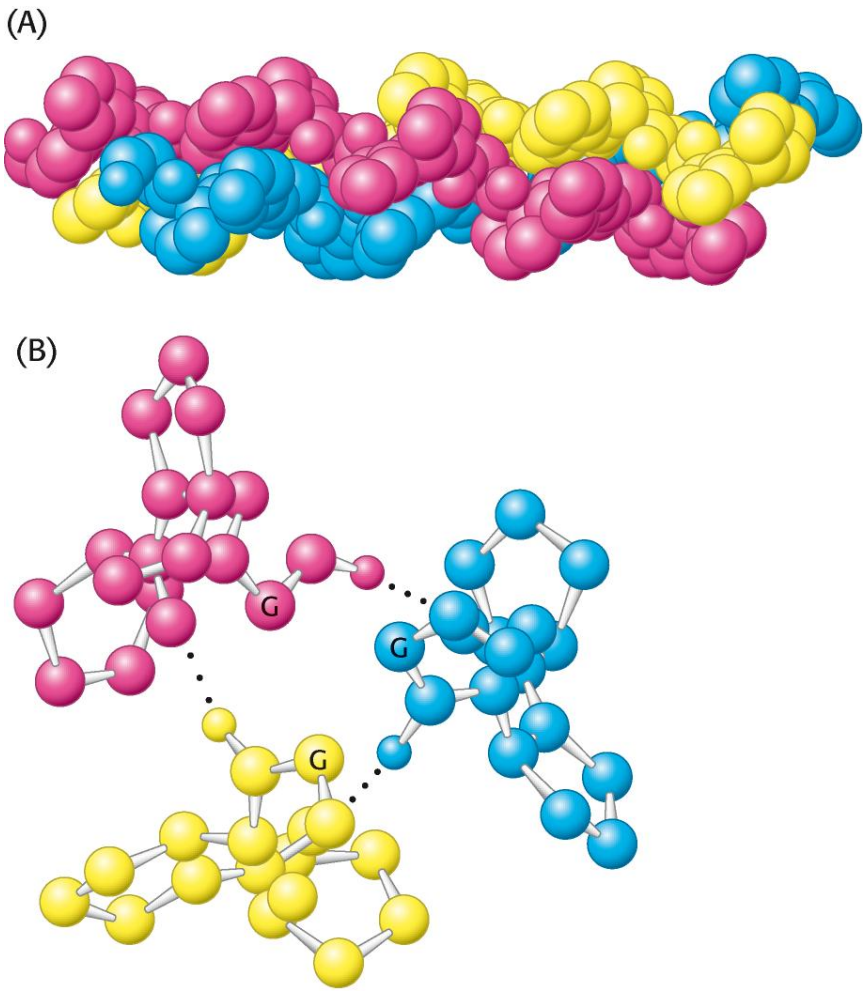
IL COLLAGENE

Sequenza ripetuta: Gly- X – Pro/OHPro, elica sinistrorsa con 3 residui per giro

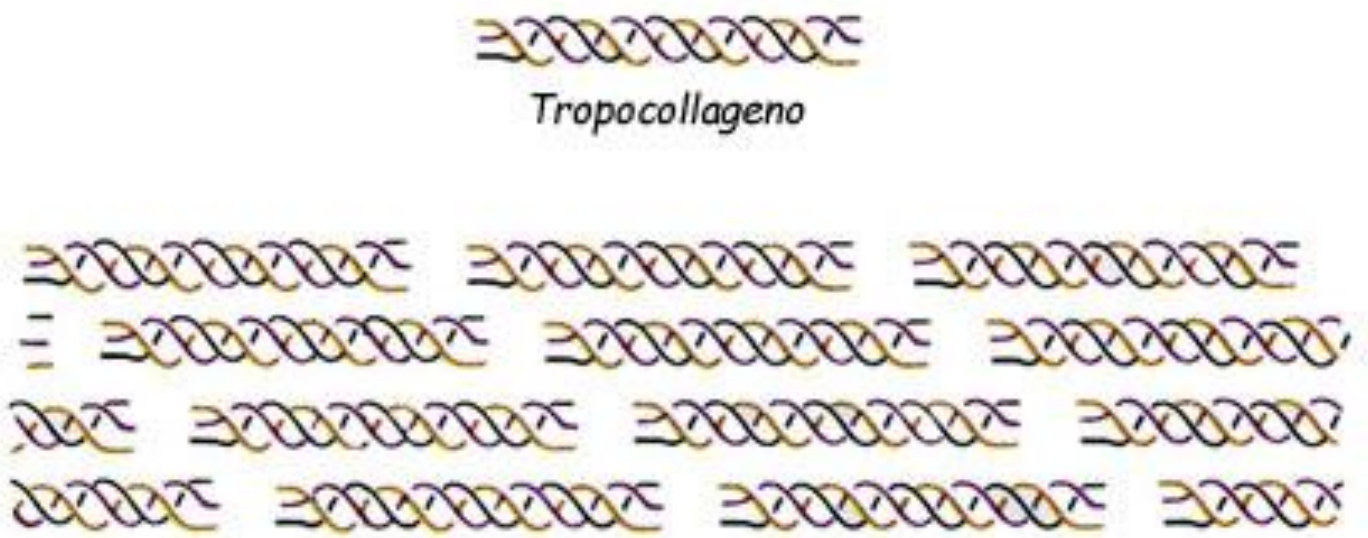
13
-Gly-Pro-Met-Gly-Pro-Ser-Gly-Pro-Arg-
22
-Gly-Leu-Hyp-Gly-Pro-Hyp-Gly-Ala-Hyp-
31
-Gly-Pro-Gln-Gly-Phe-Gln-Gly-Pro-Hyp-
40
-Gly-Glu-Hyp-Gly-Glu-Hyp-Gly-Ala-Ser-
49
-Gly-Pro-Met-Gly-Pro-Arg-Gly-Pro-Hyp-
58
-Gly-Pro-Hyp-Gly-Lys-Asn-Gly-Asp-Asp-



FILAMENTO SUPERELICOIDALE DEL COLLAGENE (tropocollagene)
Interno dell'elica molto compatto, stabilizzato da legami H intercatena
Filamento stabilizzato da repulsione steriche tra anelli pirrolidinici Pro



FIBRE DEL COLLAGENE:
unità di tropocollageno che si dispongono parallelamente, in maniera sfalsata, uniti da legami crociati tra Lys



Proteine multiadesive della matrice

Lunghe molecole flessibili che contengono domini di legame per:

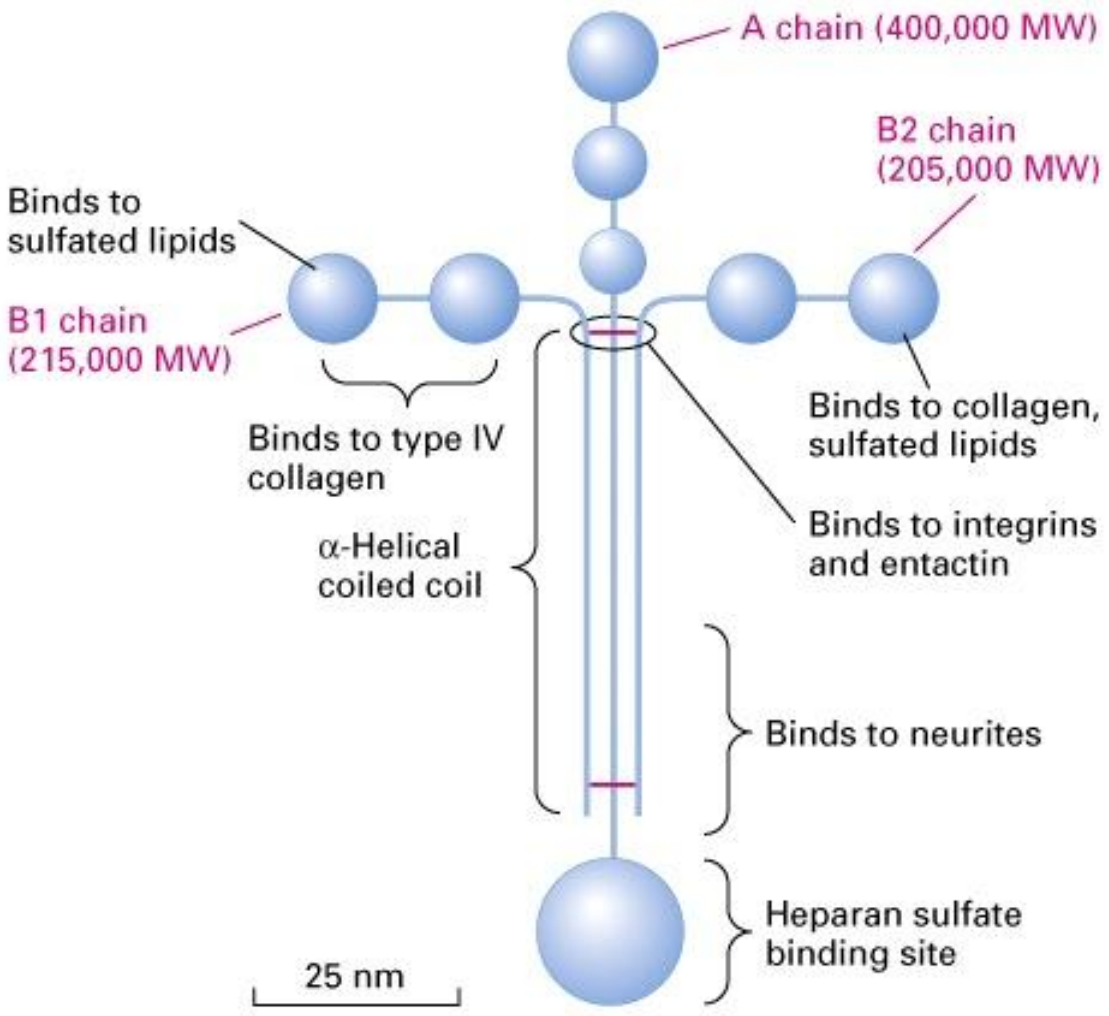
- Collagene
- Altre proteine della matrice
- Polisaccaridi
- Proteine localizzate sulla superficie delle cellule
- molecole segnale (GF)

Laminina e Fibronettina

LAMININA

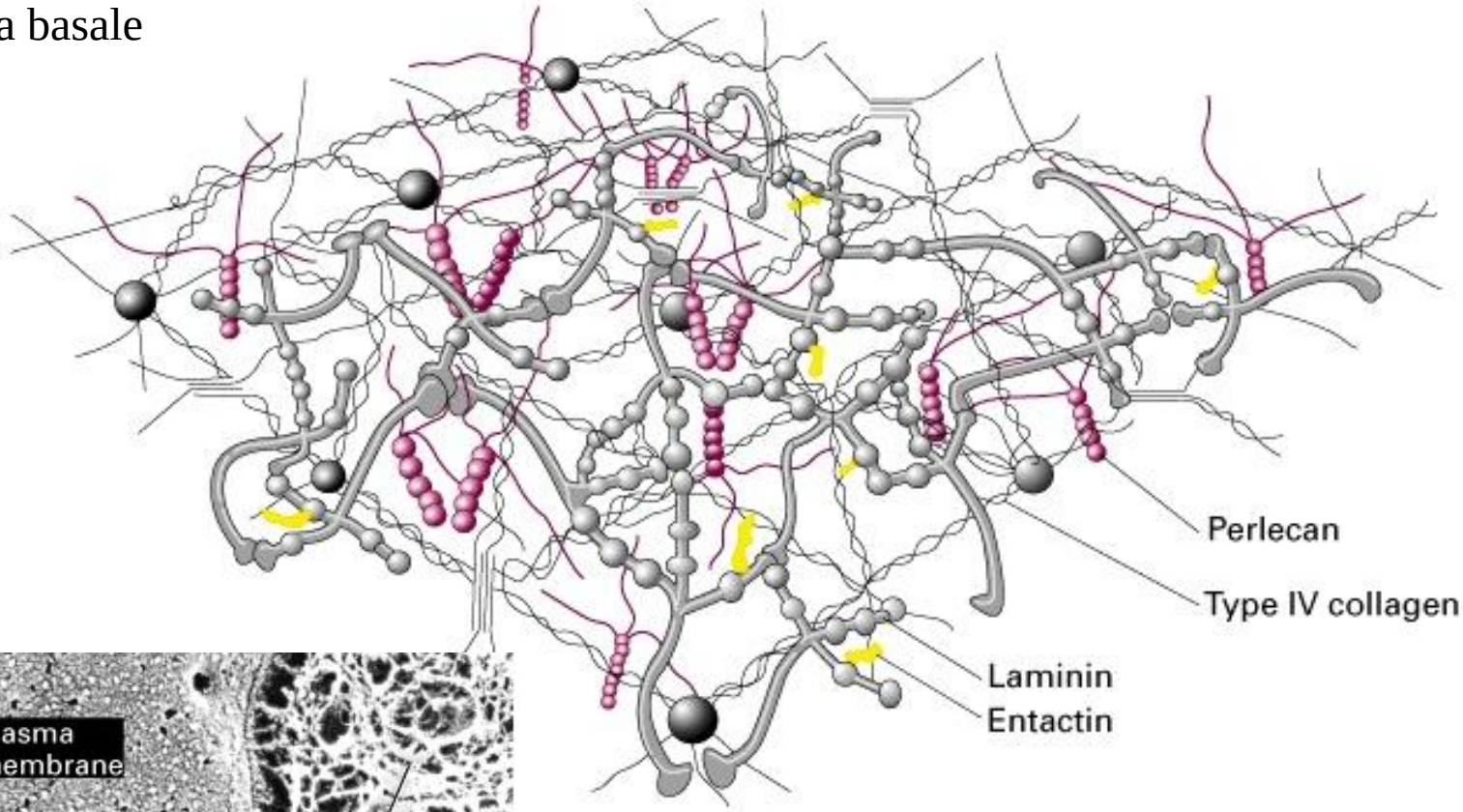
-uno dei principali costituenti delle membrane (lamine) basali, insieme al collagene IV

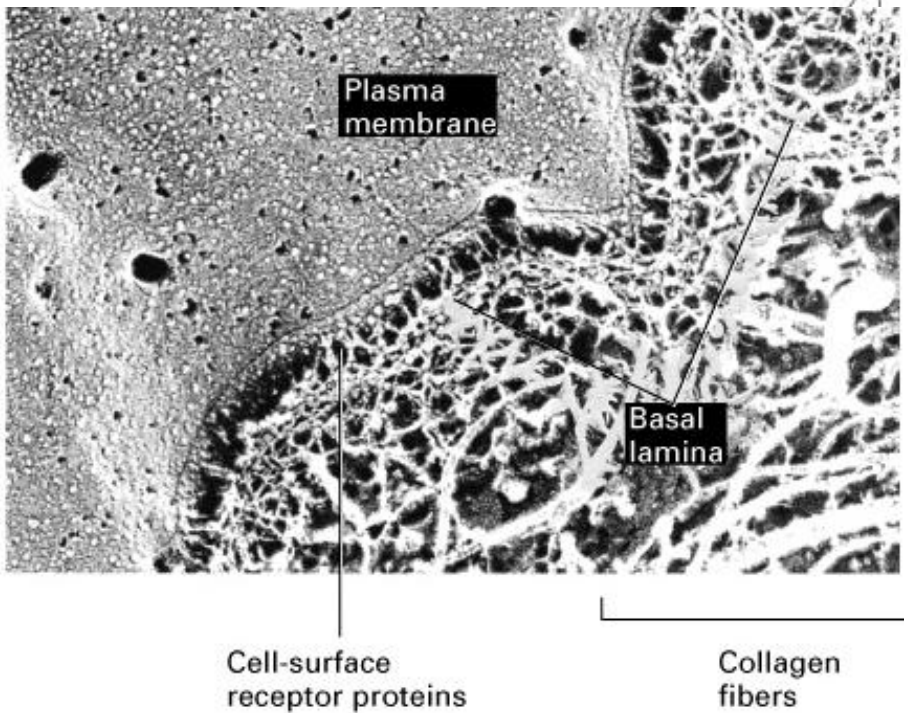
-uno dei principali fattori di regolazione dello sviluppo degli strati epiteliali



Eterotrimero di ≈ 800 kDa!

Modello di lamina basale



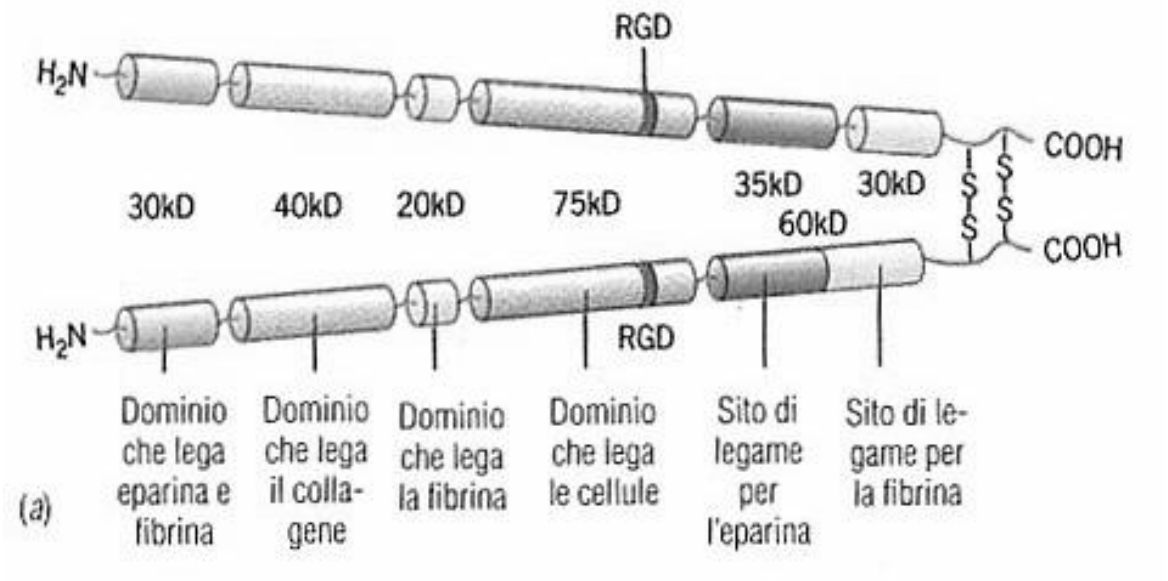


Perlecano: proteoglicano contenente eparan solfato

Entactina: proteina multiadesiva

FIBRONETTINA

Dimero circolante formato da due subunità di ~ 275 kD, unite da due ponti disolfuro



FIBRONETTINA

• fa aderire le cellule alla ECM attraverso il legame con le integrine presenti sulla membrana cellulare;

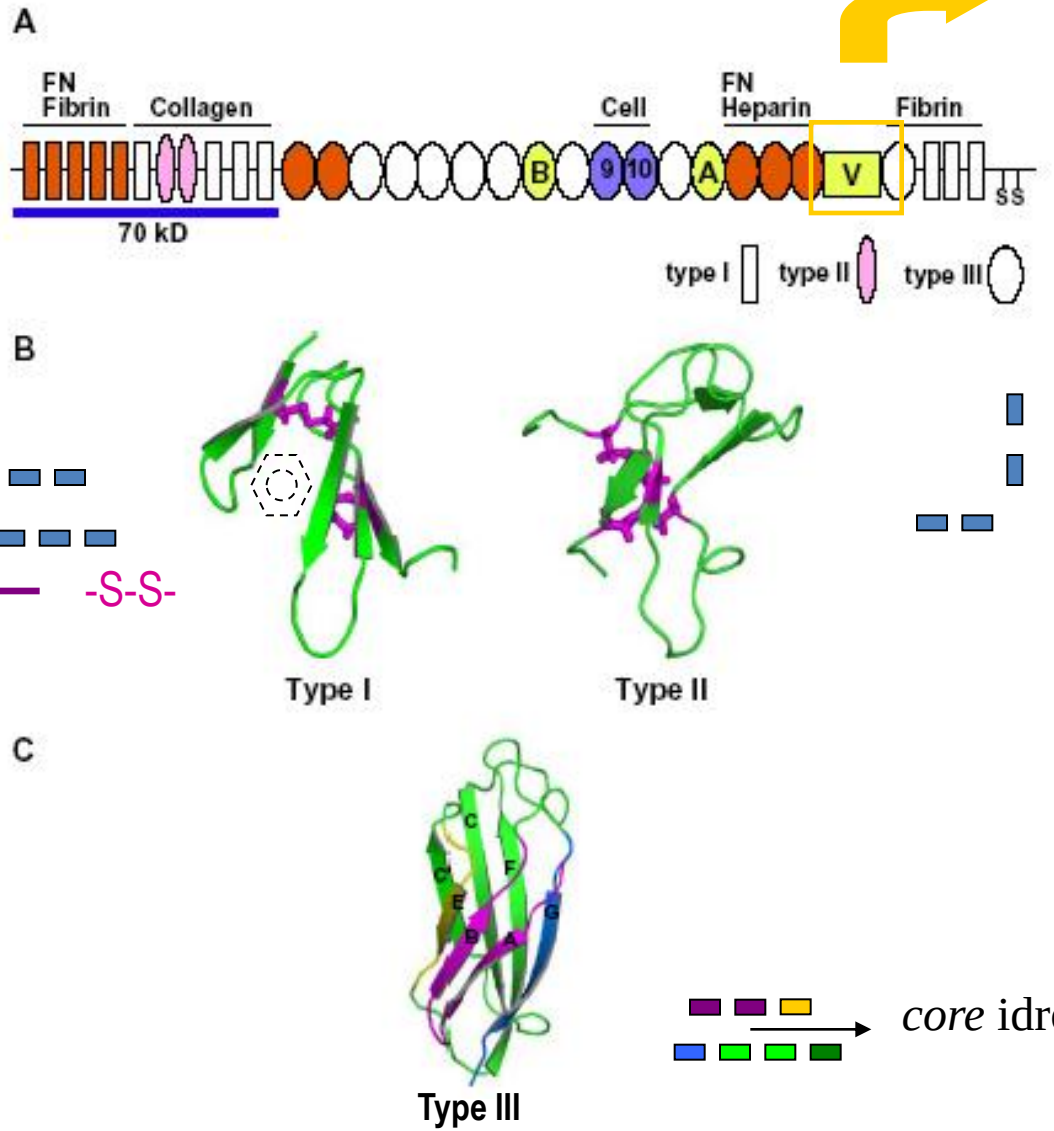
• controlla la forma delle cellule e l'organizzazione del citoscheletro;

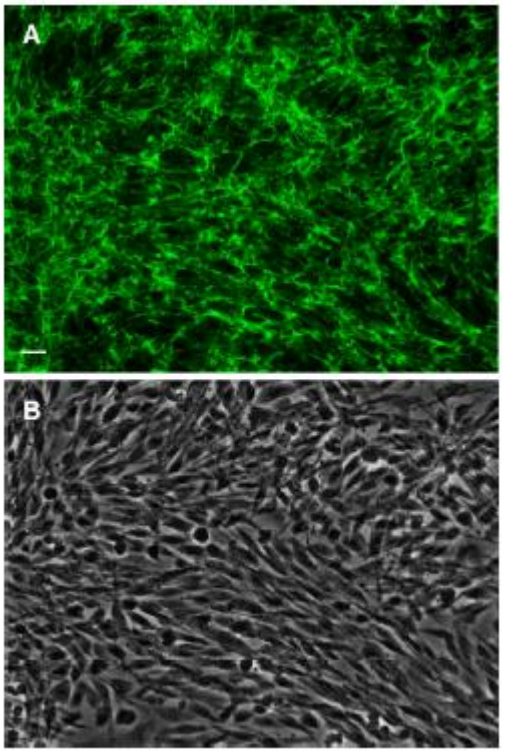
• interagisce inoltre con il collagene, la fibrina e l'eparina presenti nell'ECM.

Ogni subunità contiene 3 tipi di moduli ripetuti:
•Tipo I (12)
•Tipo II (2)
•Tipo III (15-17)

FIBRONETTINA

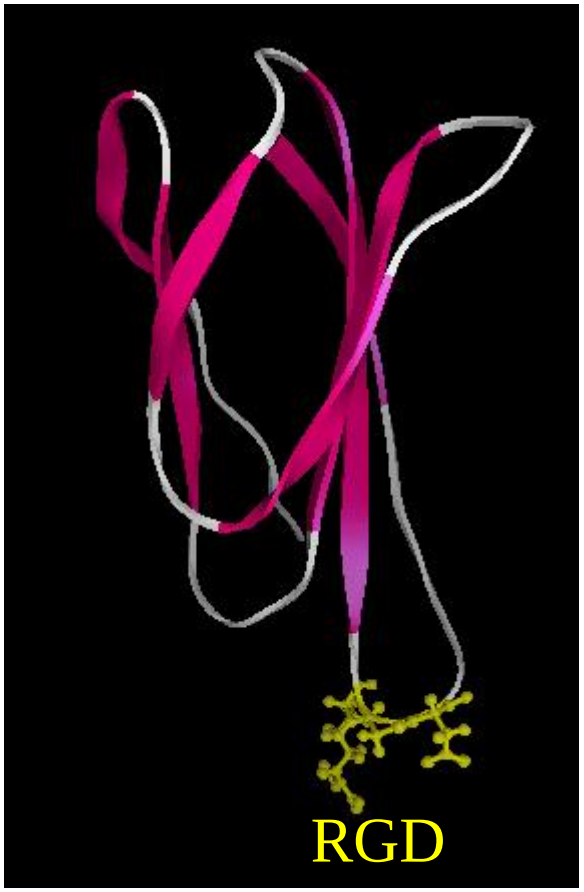
Essenziale per la secrezione





core idrofobico

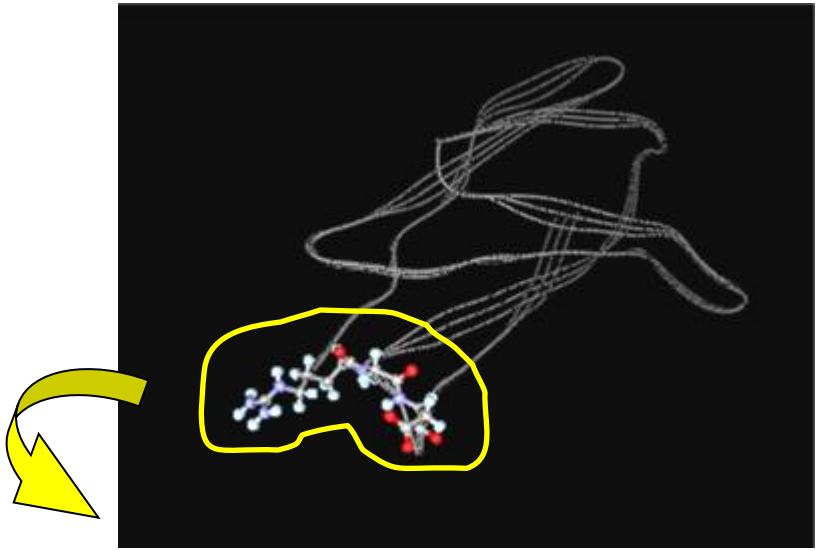
Dominio di tipo III della fibronettina

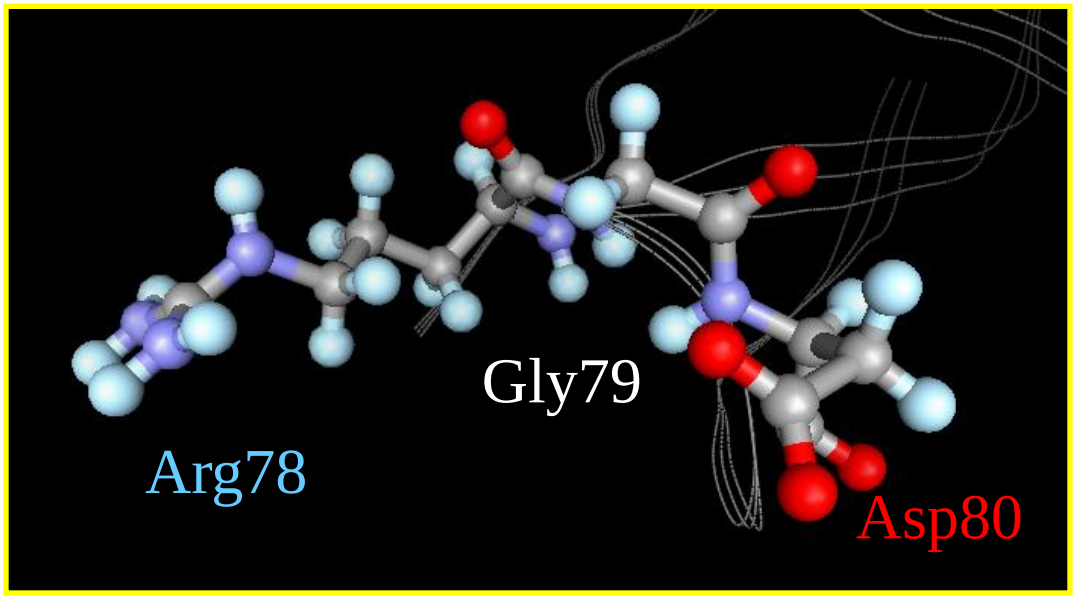


7 filamenti β

↓

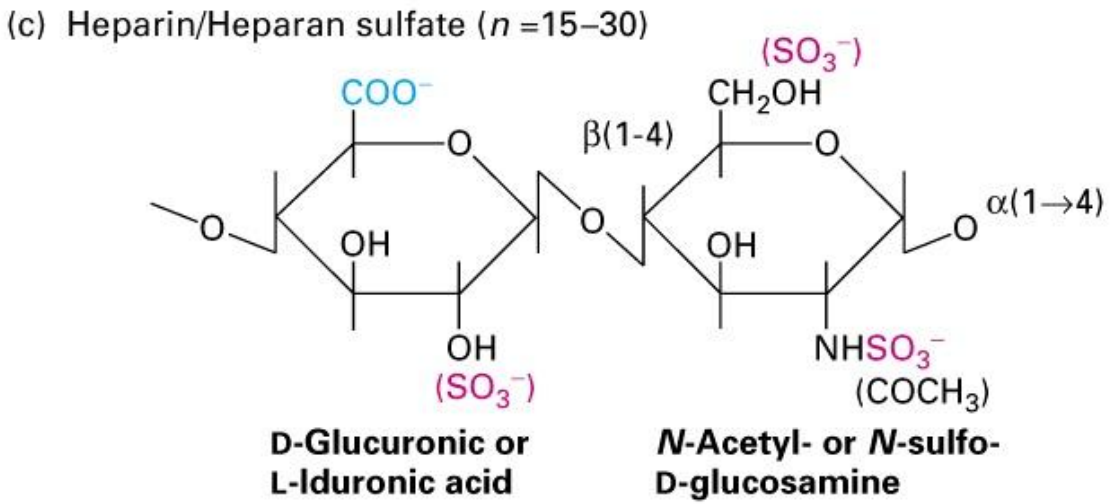
2 foglietti β antiparalleli





GLUCOSAMMINOGLICANI (GAG)

Lunghi polimeri lineari di disaccaridi

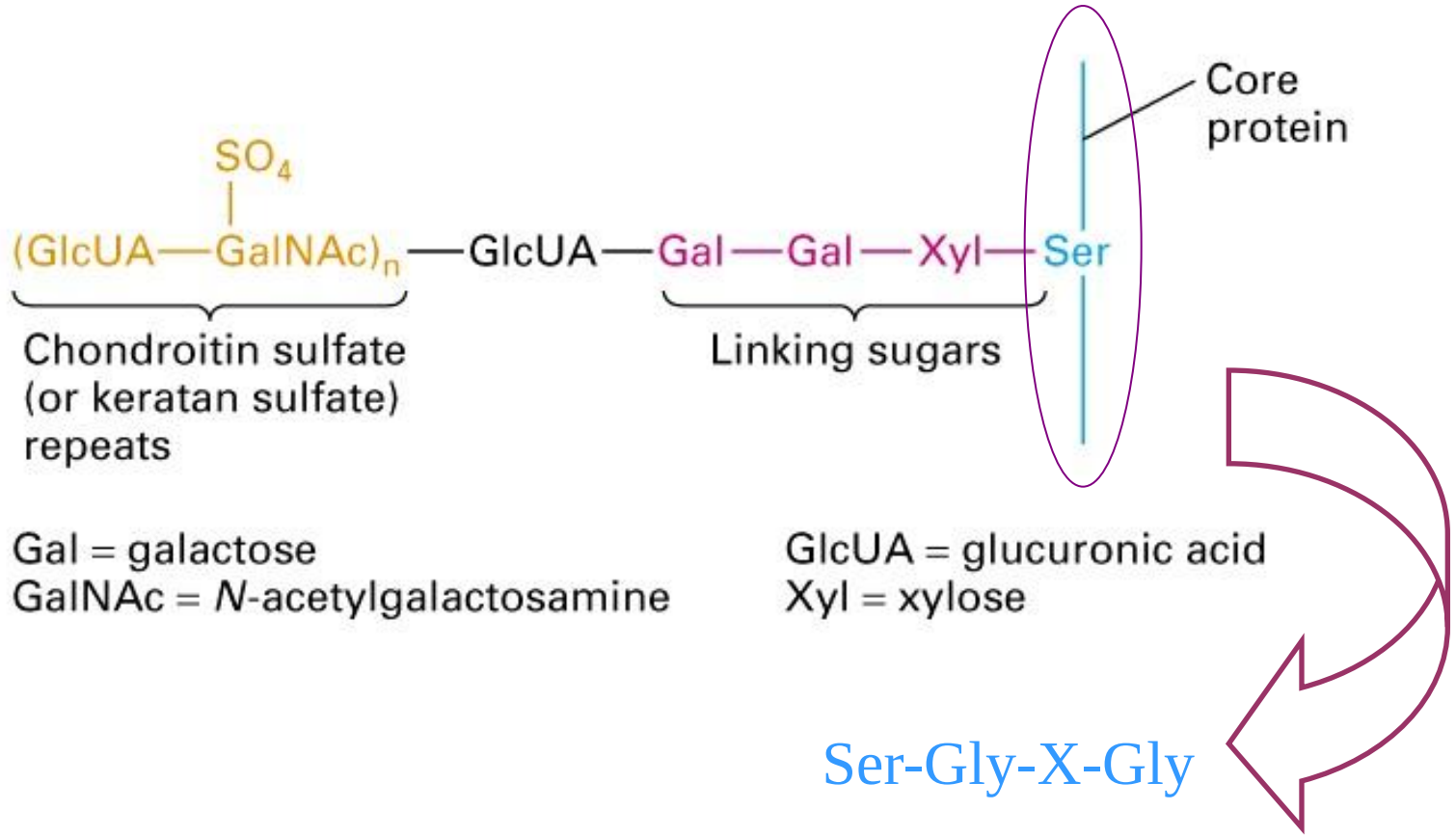


-Presentano molte cariche negative

-possono complessare con numerose molecole di H_2O, formando dei veri e propri gel che possono rendere più efficiente il legame di fattori di crescita ai loro recettori (FGF), danno volume all'ECM, e formano dei veri e propri setacci molecolari

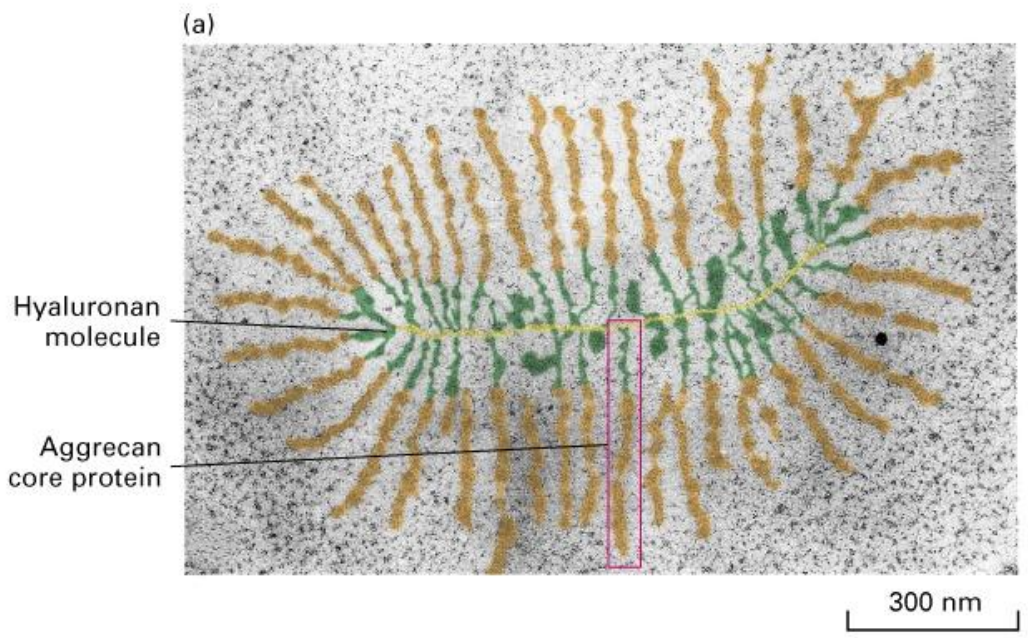
Proteoglicani

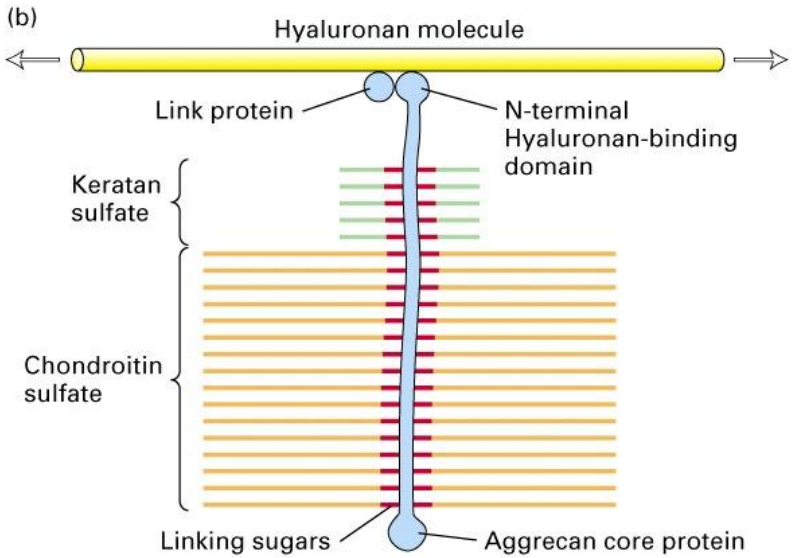
costituiti da ripetizioni di glicosamminoglicani legati a una catena polipeptidica



Proteoglicani

Possono essere componenti dell'ECM:



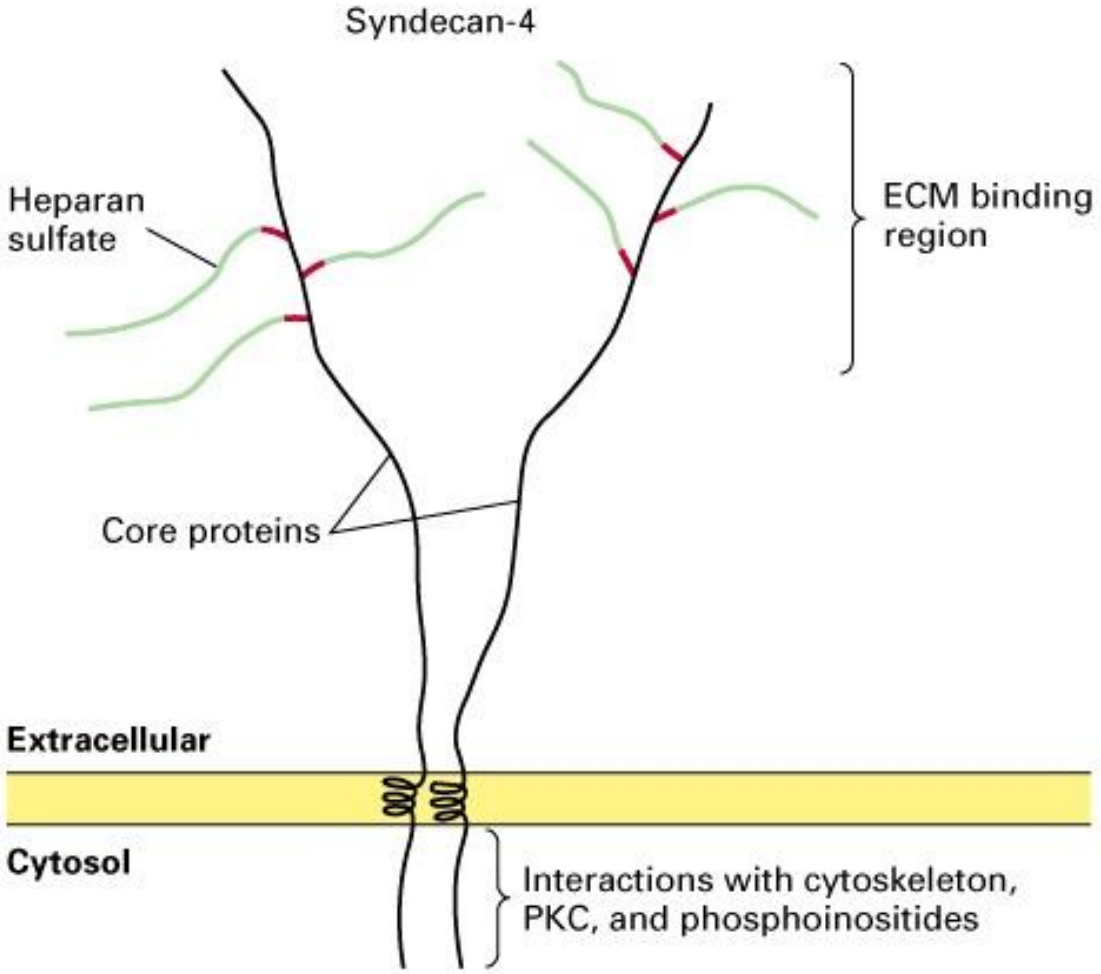


Aggregati di aggrecano della cartilagine

resistenza alla compressione
migrazione

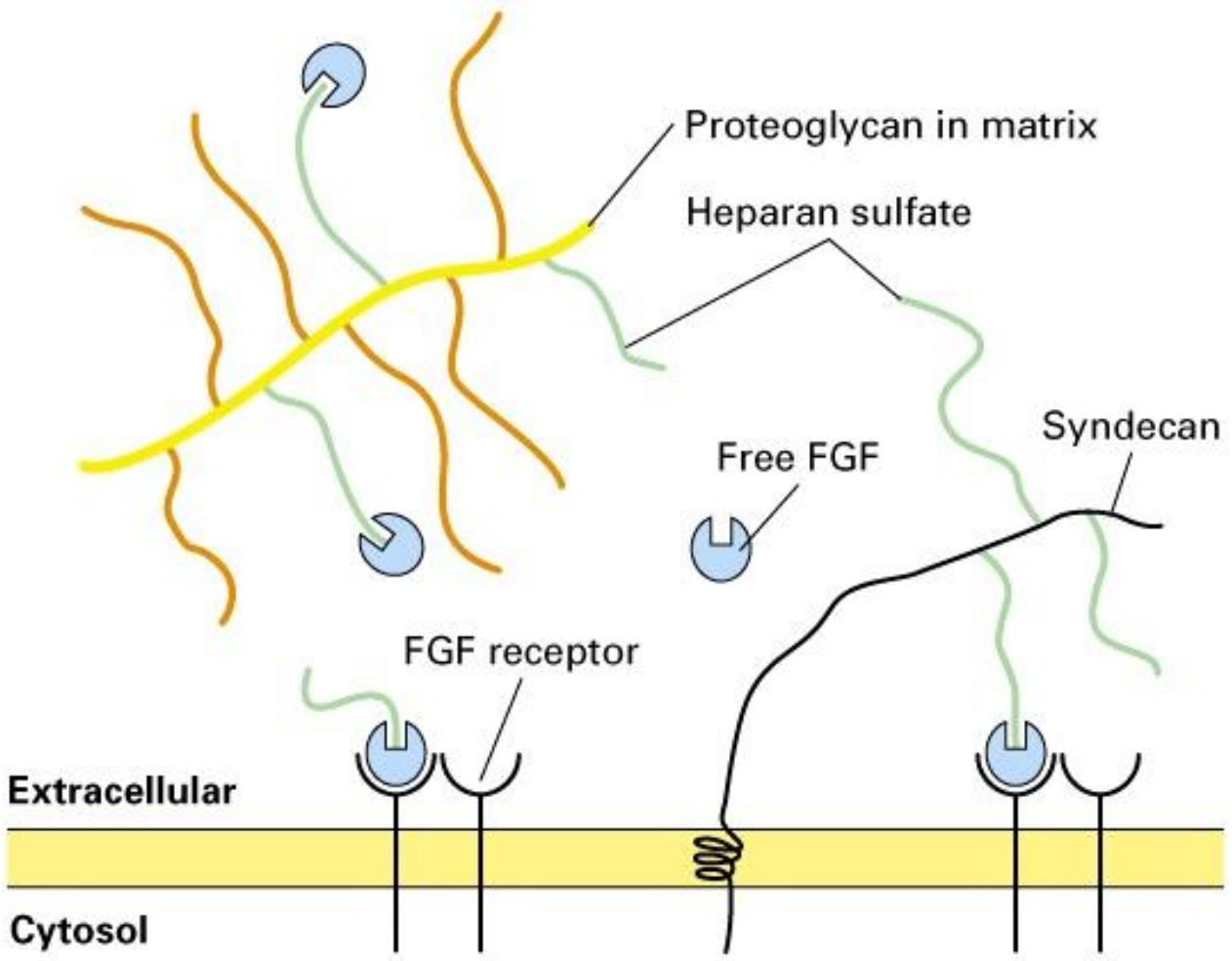
Proteoglicani

Possono essere proteine di membrana (cell. epiteliali):



Collageni fibrosi
fibronectina

Nel caso di FGF, i proteoglicani di membrana e dell'ECM cooperano nel direzionare il fattore al suo recettore



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