Insegnamento

Biologia Cellulare Avanzata

(6 CFU)

(Maria **ISABEL** Buceta Sande de **FREITAS**)

http://www-3.unipv.it/webbio/anatcomp/freitas/freitas.html

Laurea Magistrale
in Biologia Sperimentale ed Applicata
Curriculum **Scienze Biomediche Molecolari**

Presentazione del Corso

http://www-3.unipv.it/webbio/anatcomp/freitas/freitas.html

Anno Accademico 2015-2016

- Modulo di **BIOLOGIA DELLA CELLULA ANIMALE (6 CFU)**. Insegnamento di Biologia della Cellula Animale - Biologia della Cellula Vegetale (9 CFU) (1° Anno, CL triennale in Biotecnologie; Gruppi A e B) (1° Semestre).
- Insegnamento: **BIOLOGIA CELLULARE AVANZATA (6 CFU)** (1° Anno, Curriculum Scienze Biomediche Molecolari, Laurea Magistrale in Biologia Sperimentale e Applicata (2° Semestre).

Programma

- Approfondimenti sulla struttura e ruolo della ***membrana plasmatica*** nel riconoscimento tra cellule e nell'adesione cellula/cellula e cellula/matrice. Compartimentazione e dinamica dei microdomini di membrana: "***rafts" lipidici.***
- ***Microvescicole extracellulari*** (exosomi, ectosomi, ecc.) e loro ruolo nella comunicazione cellulare
- ***Molecole di adesione:*** funzione, collegamento con il citoscheletro e con la matrice extracellulare, ruolo nella trasduzione di segnali "outside-in" e "inside-out".
- ***Matrice extracellulare*** (MEC): composizione, importanza della struttura multimodulare delle (glico)proteine della MEC, dinamica della MEC (sintesi, elaborazione, degradazione con particolare attenzione alle proteasi e inibitori delle proteasi); matrichine ad effetto paracrino e juxtacrino. Esempi di matrichine con ruolo antiangiogenico. Analisi degli argomenti trattati nell'ambito dei processi di differenziamento e crescita tumorale.

Seminari su articoli di attualità. Osservazione al microscopio confocale di preparati fluorocromizzati.

Testi raccomandati (a scelta)

Testi in inglese

- Molecular Biology of the Cell. Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, Peter Walter. Garland Pub; 6th edition (2015) ISBN: Paperback: 978-0-8153-4464-3
- Molecular Cell Biology. H. Lodish, A. Berk, C.A. Kaiser, M. Krieger, A. Bretscher, H. Ploegh, A. Amon, M.P. Scott: W H Freeman & Co.; 7th edition (2013). ISBN-13: 978-1-4292-3413-9.

Traduzioni in italiano

- **BIOLOGIA MOLECOLARE DELLA CELLULA**, Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P: 5° ed. (2009), Zanichelli. ISBN 9788808064516 (con DVD)
- **LA CELLULA - UN APPROCCIO MOLECOLARE,** G.M. Cooper - R.E. Hausman:, 3a ed., Piccin , 2005. ISBN: 88-1768-0.
- **BIOLOGIA MOLECOLARE DELLA CELLULA** Lodish H, Berk A, Zipursky L , Matsudaira P, Baltimore D, Darnell J E , 2° ed. italiana (condotta sulla 4° ed. americana). ISBN 8808-08901-0]

+ Materiale fornito dalla docente

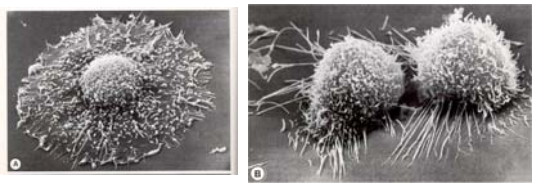
Fluidità delle membrane

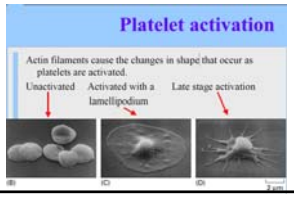
- In Biologia, il termine **fluidità di membrana** si riferisce alla **viscosità del doppio strato lipidico** di una membrana cellulare o di una membrane sintetica.
- **L'impacchettamento dei lipidi** può influenzare la fluidità della membrana.
- A sua volta la viscosità della membrane può influenzare la rotazione e la diffusione delle proteine e di altre biomolecule all'interno delle membrane, in questo modo influenzando le funzioni di queste molecule.

Microdomini lipidici (in particolare «rafts»)

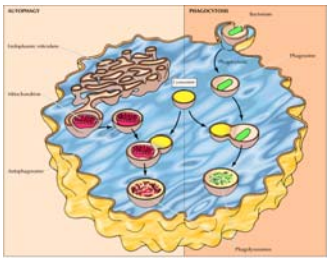
PERCHÈ È COSÌ IMPORTANTE LA FLUIDITÀ DI UNA MEMBRANA?

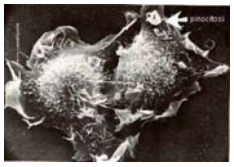
Non solo per: Plasticità forma cellula

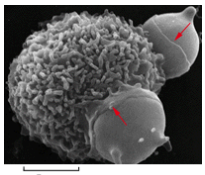




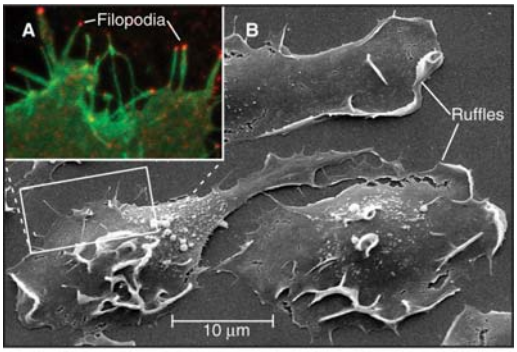
Non solo per: Pinocitosi, endocitosi, fagocitosi







Non solo per: Migrazione cellulare



La fluidità delle membrane è fondamentale per accordare finemente il comportamento delle proteine transmembrana

OILING THE WHEELS OF PROTEINS

Steven Buckingham

Evidence is emerging that the behaviour of membrane proteins is not only controlled by various signalling molecules, but also by the very types of membrane swimming around where they lie.

Gradually, however, this static view of the membrane is giving way to one in which the composition of the membrane plays an active role in fine-tuning the performance of the proteins embedded in it. "It makes sense intuitively that protein function will be affected by its membrane environment," argues Michael Caplan of Yale University. "It is like swimming in water compared with swimming in marshmallow."

HORIZON A LIVING FRONTIER

Proteine di membrana

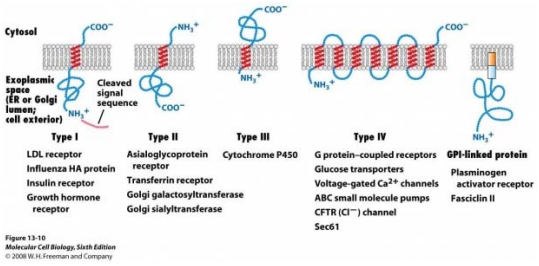
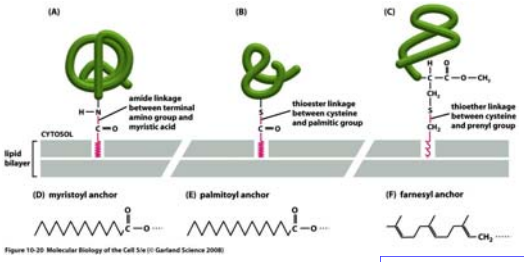


Figure 13-10
Molecular Cell Biology, Sixth Edition

http://studydroid.com/imageCards/0c/ga/card-13117662-back.jpg3.html

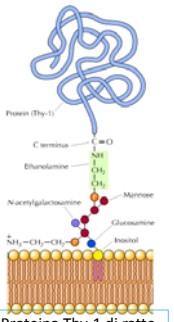
Ancore lipidiche (1)
gruppi miristoil, palmitoil e farnesil



Ancora: lipide **saturo** (si inserirà in zone riche di lipidi saturi; sfingolipidi nei rafts

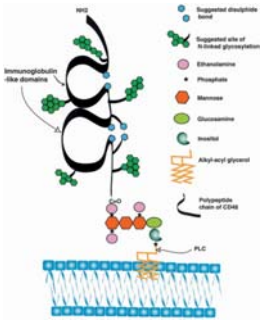
Ancora: lipide insaturo (si inserirà in zone riche di lipidi insaturi; glicerolipidi

Ancore lipidiche (2)
Glicosilfosfatidilinositolo, GPI



Proteina Thy-1 di ratto, con coda di GPI

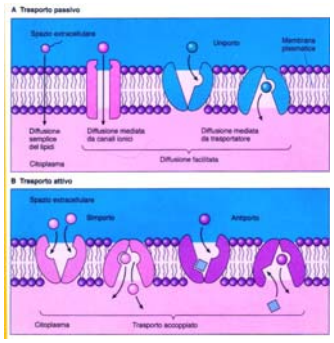
http://www.ncbi.nlm.nih.gov/books/NBK9843/figure/A1221/



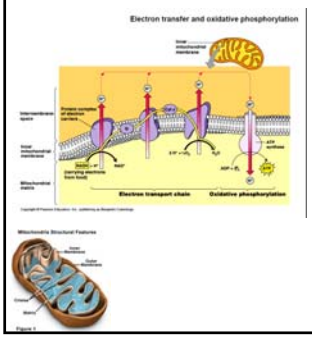
Proteina CD48 con coda di GPI

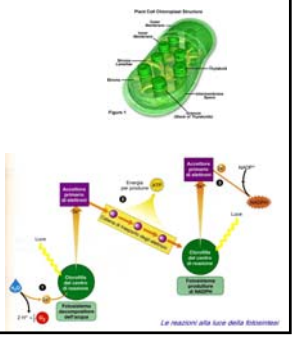
http://femsle.oxfordjournals.org/content/197/2/131

Cambiamento di conformazione dei trasportatori di membrana

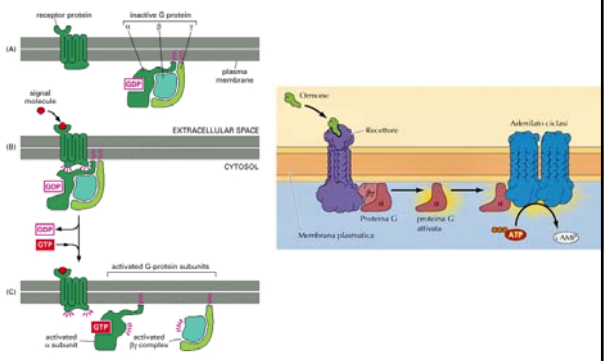


Trasporto di elettroni: fosforilazione ossidativa, fotosintesi

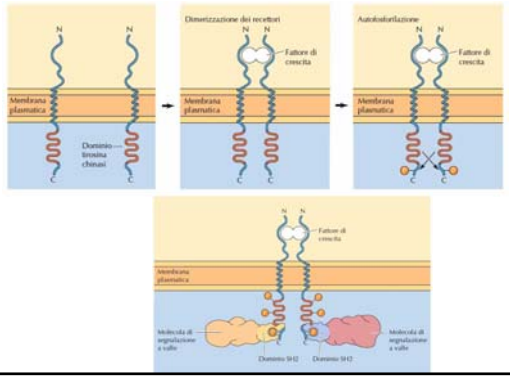


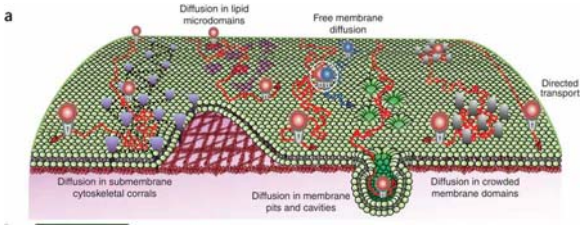


Trasduzione dei segnali (1)



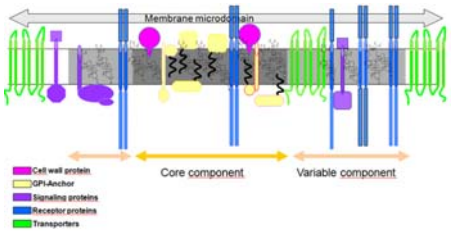
Trasduzione dei segnali (2)





Rappresentazione schematica di diverse modalità di **diffusione laterale di lipidi e proteine** della membrane plasmatica rivelate mediante **single-quantum dot tracking (SQT) technology.**

http://www.nature.com/nmeth/journal/v7/n4/fig_tab/nmeth.1444_F3.html#figure-title

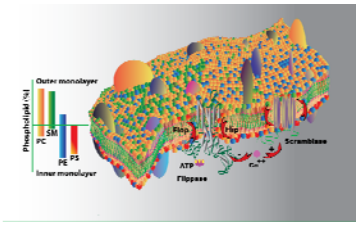


- Molte **proteine di segnalamento** funzionano come **omo**- o **eterodimeri** e ci sono diverse indicazioni che, in specifiche situazioni di **stress,** i **recettori** formino **complessi con altre proteine coinvolte nel segnalamento**.
- Inoltre, l'organizzazione delle membrane sotto forma di "**microdomini**" dipendenti dagli steroli fornisce ulteriori modalità di regolazione della densità dei recettori e della formazione di **complessi di segnalamento**.

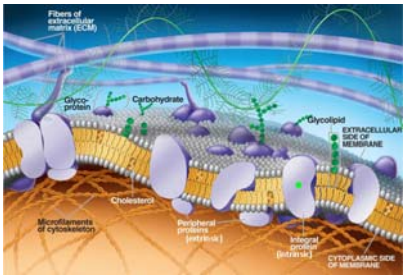
http://www.mpimp-golm.mpg.de/9376/Membrane_Microdomains_and_Protein_Complexes

The Importance of Being Earnest
by Oscar Wilde
a trivial comedy for serious people

… a lipid in a membrane



Membrana plasmatica
Aggiornamento del modello del mosaico fluido



Membrana plasmatica – Rafts lipidici

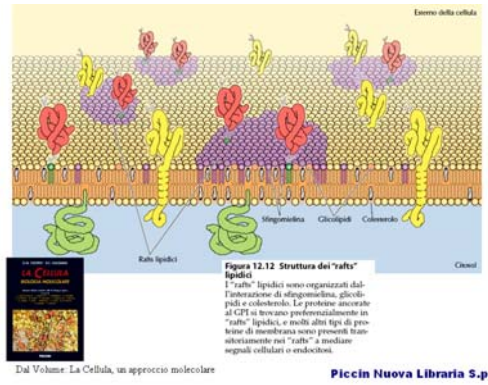
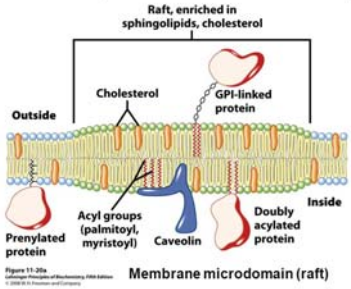


Figura 12.12 Struttura dei "rafts" lipidici
I "rafts" lipidici sono organizzati dall'interazione di sfingomielina, glicolipidi e colesterolo. Le proteine ancorate al GPI si trovano preferenzialmente in "rafts" lipidici, e molti altri tipi di proteine di membrana sono presenti transitoriamente nei "rafts" a mediare segnali cellulari o endocitosi.

Dal Volume: La Cellula, un approccio molecolare

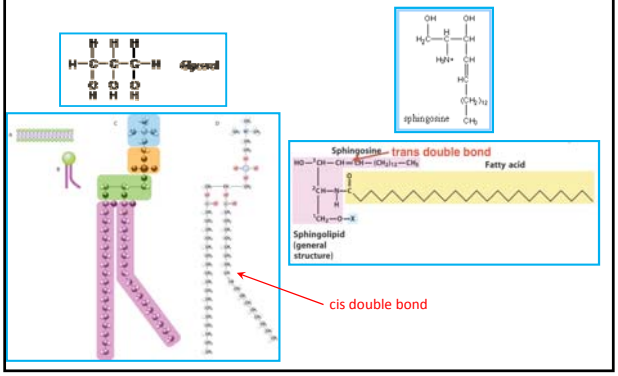
Piccin Nuova Libraria S.p.A.

Membrane composition even varies within each leaflet! (non-random distribution)

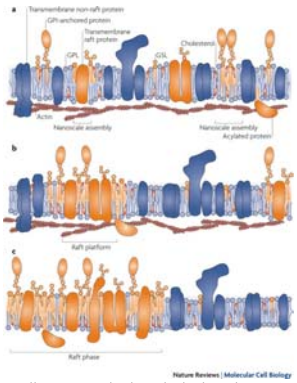


http://images.slideplayer.com/26/8711062/slides/slide_9.jpg

Membrana plasmatica
Glicerolipidi e sfingolipidi



Eterogeneità nelle membrane cellulari basate sui "rafts"

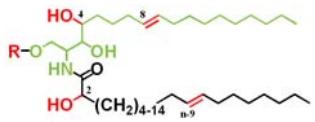


a. Gli **assemblamenti dell'ordine dei nanometri** di steroli (es. colesterolo), sfingolipidi quali la sfingomielina, glicosfingolipidi (GSLs) e proteine della membrana plasmatica hanno una composizione fluttuante. E' postulato che proteine collegate ad ancore di GPI, proteine transmembrana dei rafts e proteine citsoliche con code lipidiche aciliche siano costituenti di tali assemblamenti, che possono essere modulati da filamenti di actina. Non si sa molto sullo stato degli assemblamenti nella nanoscala del foglietto citosolico della membrana. Le proteine transmembrana non appartenenti ai rafts sono escluse da tali assemblamenti.

b. In risposta a segnali esterni o all'inizio dei processi di traffico di membrane si formano **piattaforme di rafts** a partire assemblamenti fluttuanti, mediante interazioni lipide-lipide, lipide-proteine e di oligomerizzazione proteina-proteina. Queste piattaforme sono importanti per il **segnalamento** e traffico di **membrana.**

c. Fasi di tipo **"rafts" con dimensioni dell'ordine dei micron** possono essere indotte ad uno stato di equilibrio. Questo stadio può essere visto in sistemi modello quali "giant unilamellar vesicles" (GUVs) e anche in "giant plasma membrane vesicles" (GMPVs) o in sfere di membrana plasmatica rilasciate dalla cellula. [N.B. forse ectosomi].

http://www.nature.com/nrm/journal/v11/n10/images/nrm2977-f1.jpg



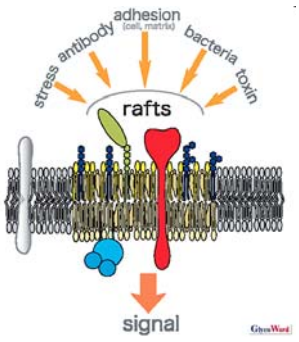
Name of sphingolipid	Name of X	Formula of X
Ceramide	—	—H
Sphingomyelin	Phosphocholine	—P(=O)(O)—O—CH2—CH2—N(CH3)3
Neutral glycolipids Glucosylceramide	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

Struttura generica di un sfingolipide
Gli sfingolipidi consistono in una base amidica a lunga catena (**verde**) collegata ad un acido grasso (**nero**). Diverse modificazioni possono avere luogo nella struttura di base (**rosso**), incluso desaturazioni (in n-4, n-8 e n-9), idrossilazioni (n-2 & n-4) e gruppo di testa (R).

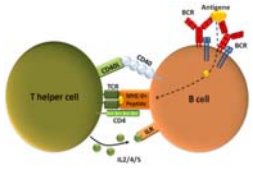
http://www.plantsphingolipids.org/wiki:sphingolipids

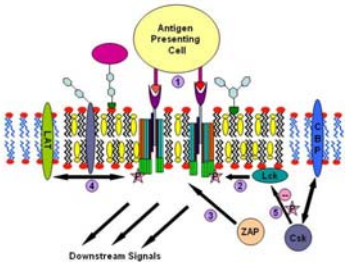
http://physwiki.ucdavis.edu/@api/deki/files/1610/table.jpg?revision=1

Lipid rafts e trasduzione di segnale

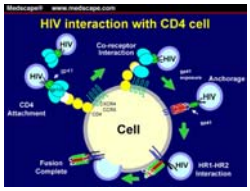


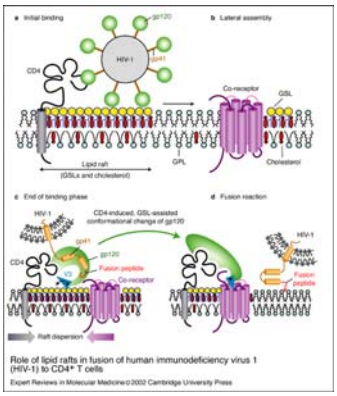
Rafts e attivazione dei linfociti



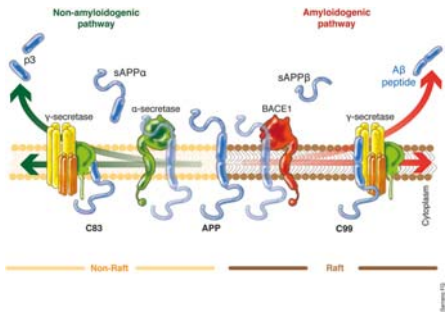


Rafts e invasione virale

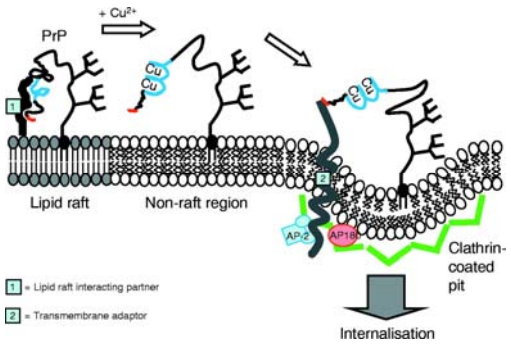




Rafts e formazione di placche di amiloide

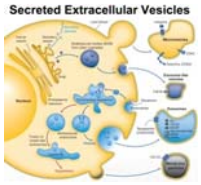


Rafts e formazione di proteina prionica



Un nuovo processo di trasmissione dell'informazione

Microvescicole extracellulari



I processi più noti di trasmissione dei segnali

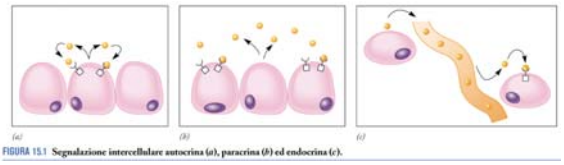
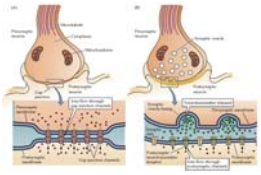
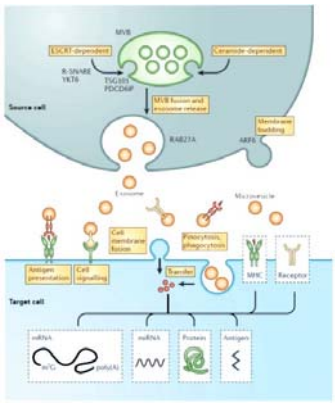


FIGURA 15.1 Segnalazione intercellulare autocrina (a), paracrina (b) ed endocrina (c).

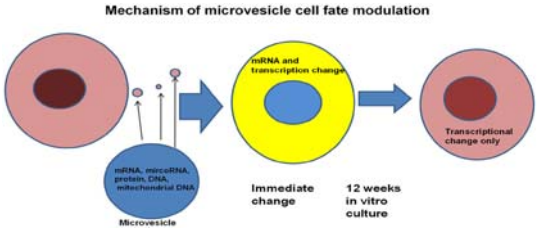


Sinapsi chimiche (gap junctions) ed elettriche



Biogenesi delle vescicole extracellulari e loro interazioni con le cellule riceventi

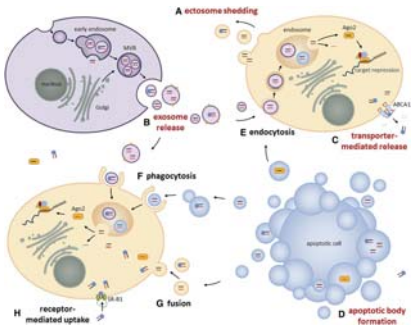
EL Andaloussi S, Mäger I, Breakefield XO, Wood MJ. **Extracellular vesicles: biology and emerging therapeutic opportunities**. Nat Rev Drug Discov. 2013 May;12(5):347-57. Fig. 1



Modulazione del destino cellulare mediante microvescicole

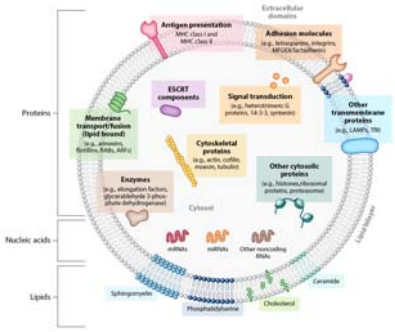
http://www.frontiersin.org/files/Articles/66136/fonc-04-00056-HTML/image_m/fonc-04-00056-g014.jpg

Rilascio e cattura di exosomi

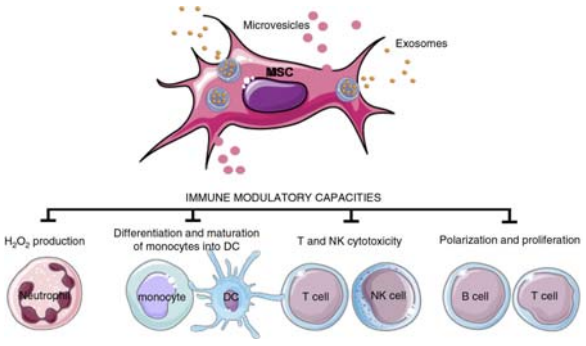


http://www.bloodjournal.org/content/bloodjournal/121/25/4977/F1.large.jpg?sso-checked=true

Contenuto delle microvescicole

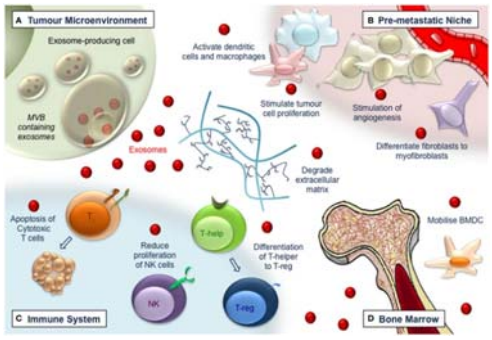


Colombo, Raposo and Théry, *Ann Rev Cell Dev Biol* 2014



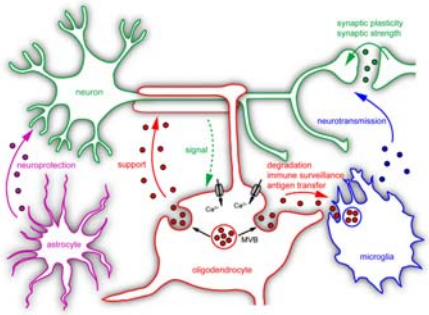
http://www.journalofextracellularvesicles.net/index.php/jev/article/viewFile/27066/html_16/160279

Microvescicole & crescita tumorale



http://www.exosome-rna.com/wp-content/uploads/2014/06/tumor.jpg

Microvescicole & Sistema nervoso



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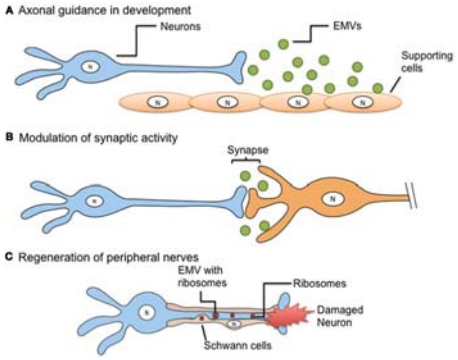

Sistema nervoso

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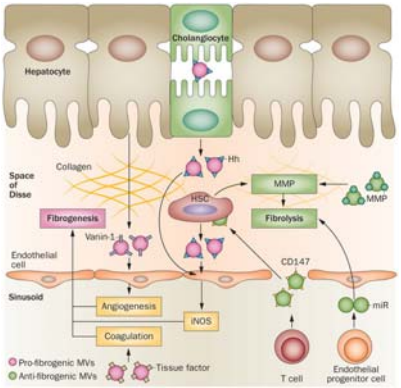
Fegato



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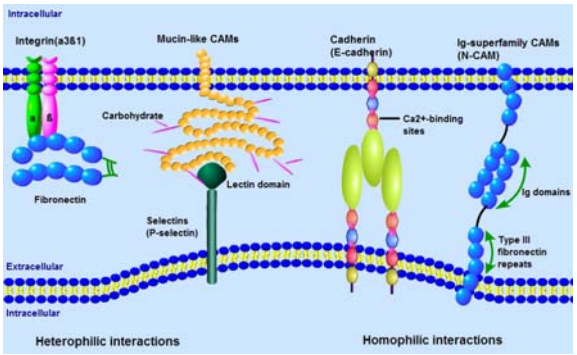
Molecole di adesione



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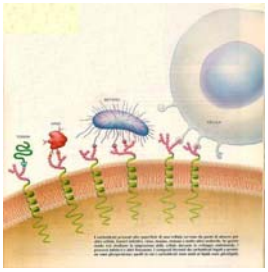
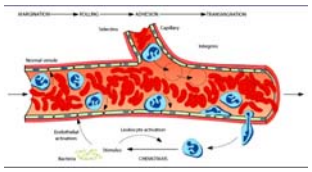
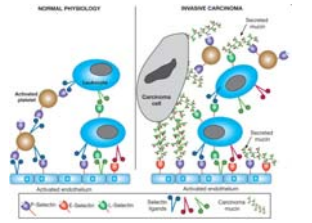
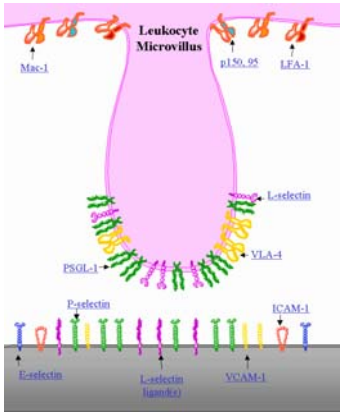
Molecole di adesione, segue



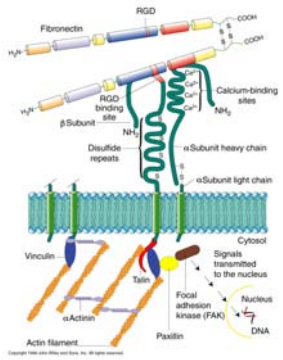




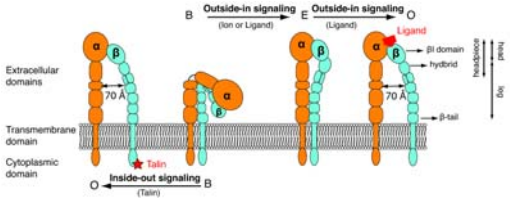
Adesione



Mol. Adesione, segue



Integrine e segnalamento



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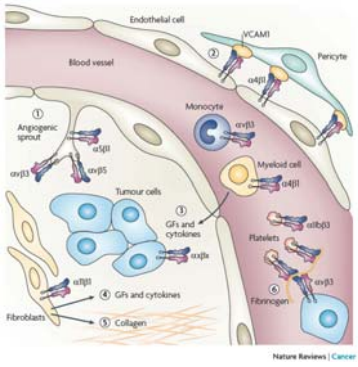
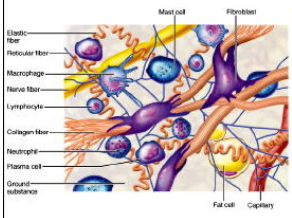
Integrine & Cancro



http://www.nature.com/nrc/journal/v10/n1/images/nrc2748-f3.jpg

ECM: Definition and Function

The acellular material around cells is called extracellular matrix (ECM)



Function:

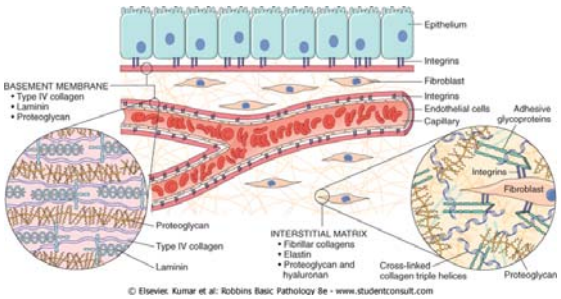
- Mechanical support for cells and tissues.
- Integrates cells into tissues.
- Influences cell shape and cell movement.
- Influences cell development and differentiation.
- Coordinates cellular functions through signaling with cellular adhesion receptors.
- Reservoir for extracellular signaling molecules.

Structure of ECM:

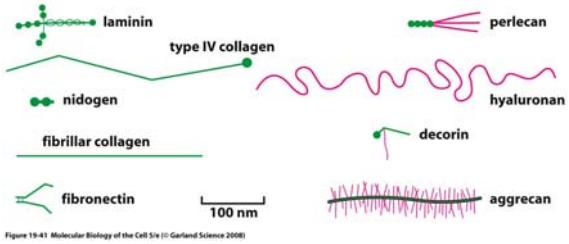
- Fibers: collagen and elastin, which provide strength and flexibility.
- Proteoglycans: protein-saccharide complexes, providing a voluminous matrix.
- Adhesive glycoproteins: 'glue' cells and ECM, e.g. fibronectin and laminin.

Extracellular Matrix, 01/07, Boris Hinz, PhD,EPFL/ISB/IPMC/LCB — Slide 2

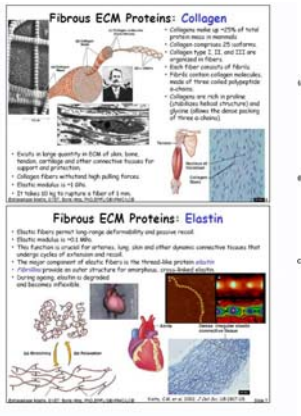
Matrice extracellulare

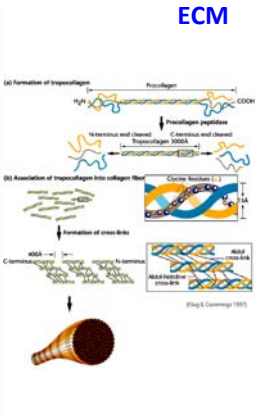


ECM

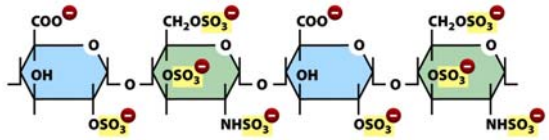


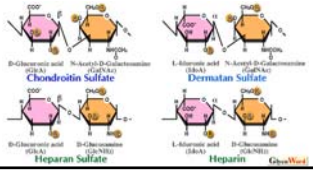
ECM



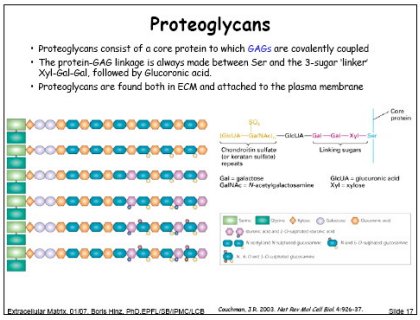


ECM - GLICOSAMINOGLICANI

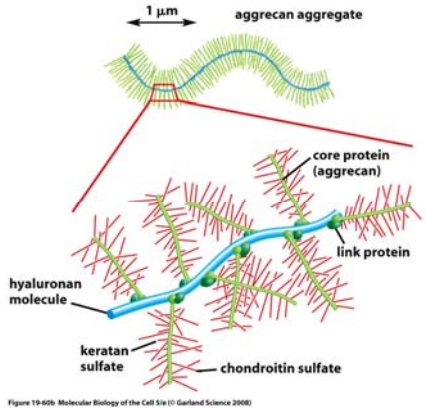




PROTEOGLICANI



ECM proteoglicani



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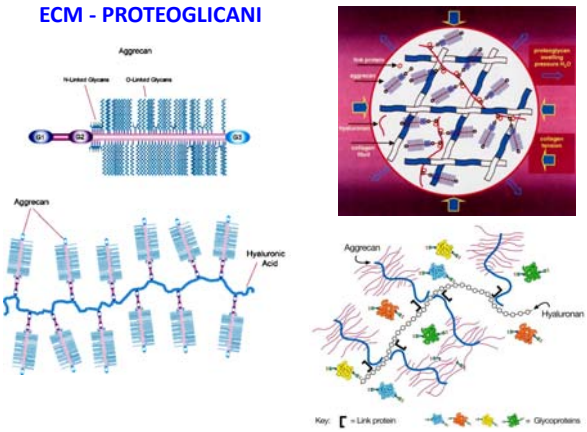
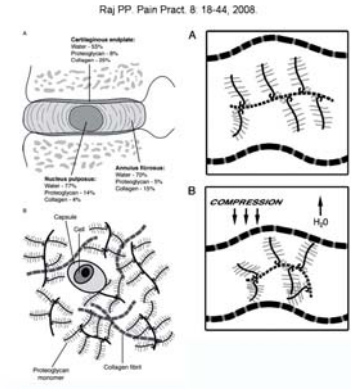
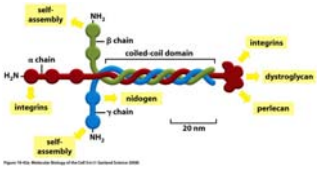


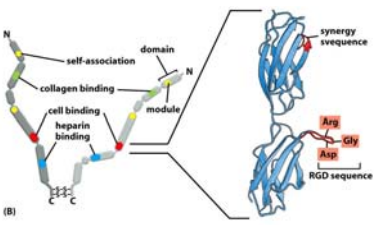
ECM - CARTILAGINE



ECM - Glicoproteine

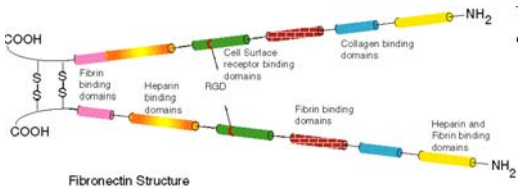


Laminina



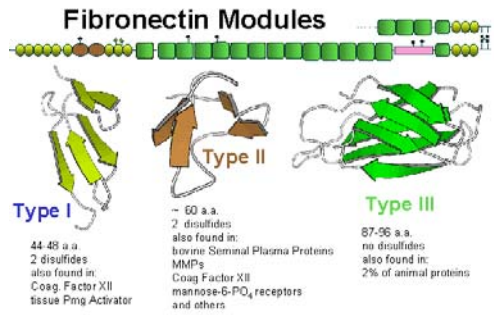
Fibronectina

FIBRONECTINA

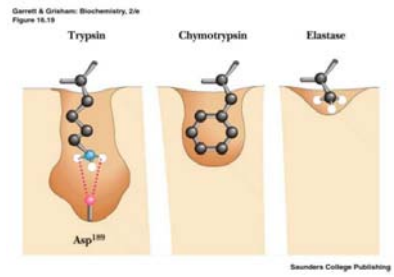


Fibronectin Structure

http://course1.winona.edu/sberg/308s08/Lec-note/Extracellular.htm



Proteasi della matrice
Serina proteasi



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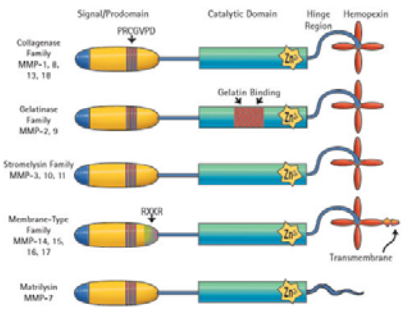
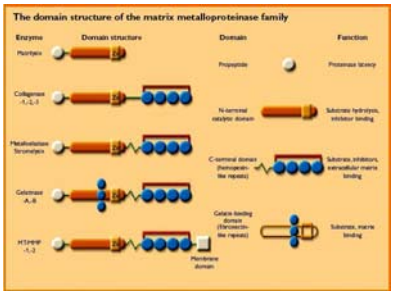
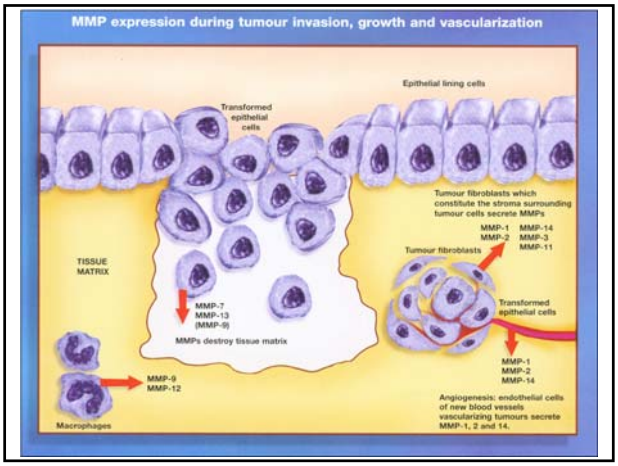
Proteasi della matrice
METALLOPROTEASI

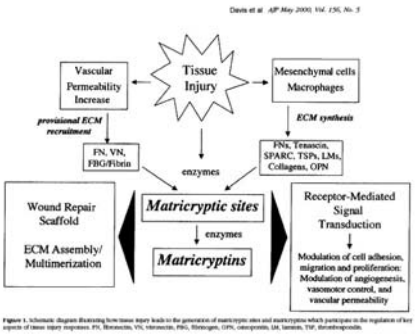


Struttura a domini delle MMPs

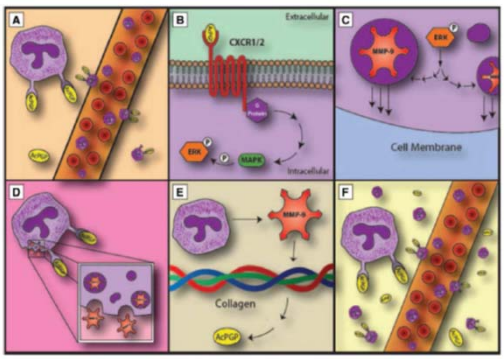




ECM - Matrichine

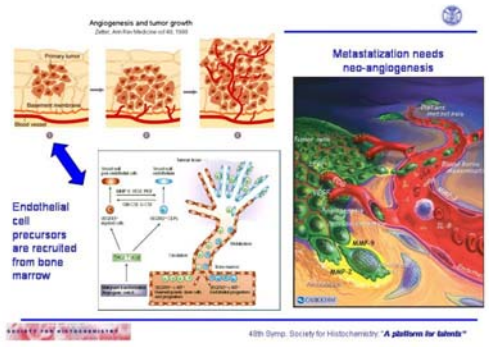


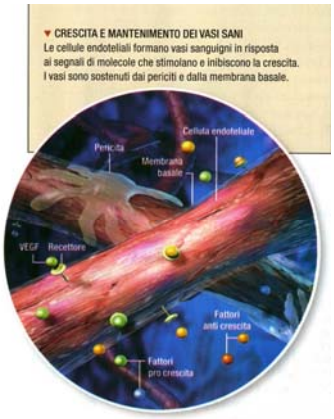
A model of persistent matrikine production and neutrophilic inflammation

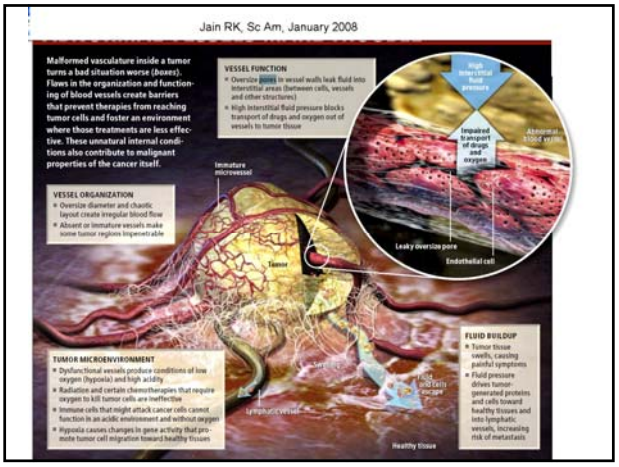


Wells JM, Gaggar A, Blalock JE. MMP generated matrikines. Matrix Biol. May-Jul;44-46:122-9. 2015

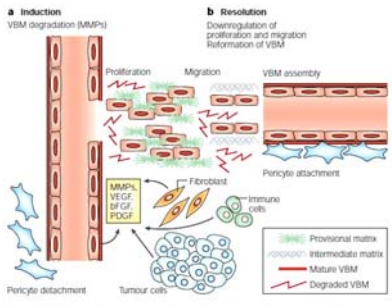
ANGIOGENESI TUMORALE



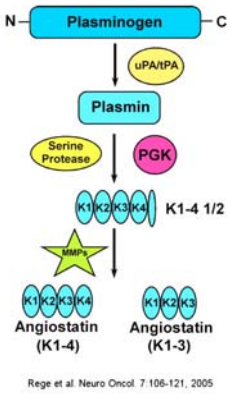




MODIFICAZIONI DELLA ECM DURANTE L'ANGIOGENESI



Inibitori dell'angiogenesi (matrichine)



Rege et al. Neuro Oncol. 7:106-121, 2005

Presentazione corso

DIAPOSITIVE AGGIUNTIVE

expert reviews

Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases

Jacques Fantini, Nicolas Garmy, Radhia Mahfoud and Nouara Yahi

The fluid mosaic model of the plasma membrane has evolved considerably since its original formulation 30 years ago. Membrane lipids do not form a homogeneous phase consisting of glycerophospholipids (GPLs) and cholesterol, but a mosaic of domains with unique biochemical compositions. Among these domains, those containing sphingolipids and cholesterol, referred to as membrane or **lipid rafts**, have received much attention in the past few years. Lipid rafts have unique physicochemical properties that direct their organisation into liquid-ordered phases floating in a liquid-crystalline ocean of GPLs. These domains are resistant to detergent solubilisation at 4 degrees C and are destabilised by cholesterol- and sphingolipid-depleting agents. Lipid rafts have been morphologically characterised as small membrane patches that are tens of nanometres in diameter. Cellular and/or exogenous proteins that interact with lipid rafts can use them as transport shuttles on the cell surface. Thus, rafts act as molecular sorting machines capable of co-ordinating the spatiotemporal organisation of signal transduction pathways within selected areas ('signalosomes') of the plasma membrane. In addition, rafts serve as a portal of entry for various pathogens and toxins, such as human immunodeficiency virus 1 (HIV-1). In the case of HIV-1, raft microdomains mediate the lateral assemblies and the conformational changes required for fusion of HIV-1 with the host cell. Lipid rafts are also preferential sites of formation for pathological forms of the prion protein (PrPSc) and of the [beta]-amyloid peptide associated with Alzheimer's disease. The possibility of modulating raft homeostasis, using statins and synthetic sphingolipid analogues, offers new approaches for therapeutic interventions in raft-associated diseases.

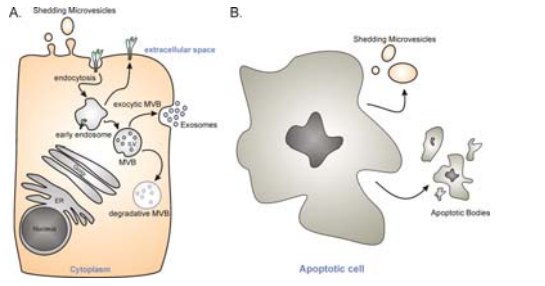
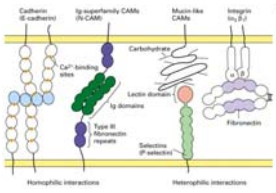
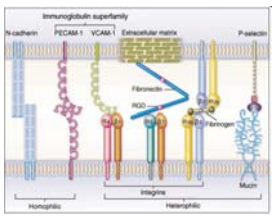


FIG. 1. Microvesicle biogenesis pathways. (A) Endocytosed proteins on the plasma membrane traffic to early endosomes where they can be sorted back to the plasma membrane or to multivesicular bodies (MVBs). MVBs contain intraluminal vesicles (ILVs) that are generated by budding from the limiting membrane of endosomes. Distinct MVB populations exist, a degradative MVB that leads to lysosomal destruction of MVB content or an exocytic pathway that traffics to the plasma membrane and, following membrane fusion, releases ILVs from the cell in the form of exosomes. Vesicles can also actively be released directly from the plasma membrane requiring a budding mechanism. These vesicles have been termed shedding microvesicles. ER, endoplasmic reticulum. (B) Dying or apoptotic cells release shedding microvesicles in the early stages of apoptosis and larger apoptotic bodies at later times that contain nuclear and cytoplasmic remnants of the degrading cell.

Meckes DG Jr, Raab-Traub N. Microvesicles and viral infection. J Virol. 2011 Dec;85(24):12844-54.

Molecole di adesione





http://labs.idi.harvard.edu/wagner/media/image1.html