

Il corso- Tecnologie per la Medicina Regenerativa

The course book: **Fondamenti di ingegneria dei tessuti per la medicina rigenerativa.** Author/s Mantero S, Remuzzi A, M.T. Raimondi, Ahluwalia A ISBN Code 978-88-55-3039-2 Publisher :Patron: Number of pages 212

- <http://www.centropiaggio.unipi.it/~ahluwalia>

(c'è un link al corso)

Portale biomedica

- www.biomedica.ing.unipi.it

Come si svolge l'esame

Orale e prova comsol

Presentazione in gruppi con questi temi

1. IPSC
2. CRISPR
3. Organoids
4. Whole organ decellularisation
5. Artificial meat
6. body on a chip
7. Organ printing
8. Artificial skin for cosmetic testing
9. GM animals and animal models (of any disease)
10. Tissue engineering (of any organ)

Esame

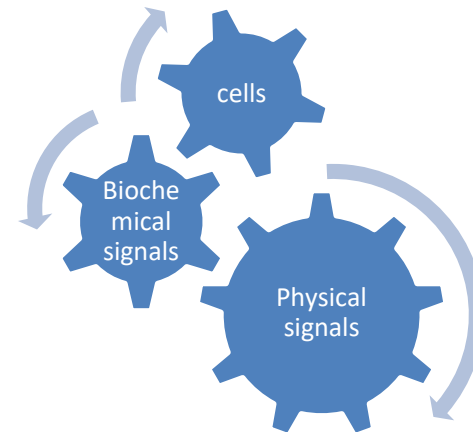
- Prova orale
- Prova comsol
- Presentazione gruppo

What is the course about?

- **Development**
- **Regenerative Medicine**
- **Tissue engineering**
- **Biomimicking tissues, organs and systems, ie. **In-vitro models** using Integrated Approaches**

Why?

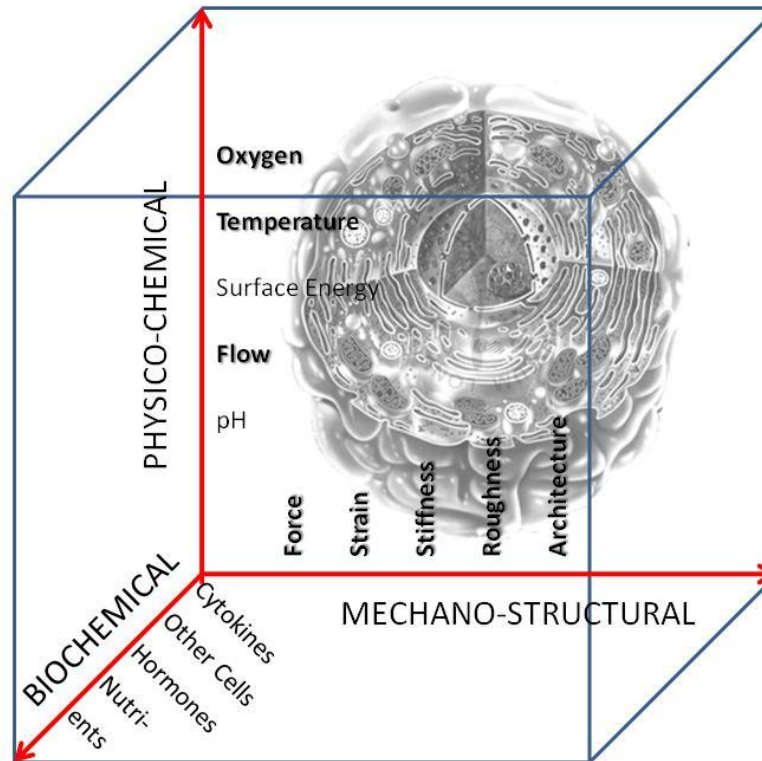
- Design downscaled biomimetic in-vitro systems for different applications
- Cell based engineering is the bioengineering of the future. —————> Biological engineering



Development: The cues of life

Stimuli

- Biochemical
- Physico-chemical
- Mechano-structural



Note even time has a role- thus a *dynamic* environment, is fundamental in all biological processes.

21 century tissue engineering (regenerative medicine)

Allopathy: a system of medical practice that aims to combat disease by use of remedies (as drugs or surgery) producing effects different from or incompatible with those produced by the disease being treated

New Regenerative medicine uses ATMP (advanced therapy medicinal products)

An ATMP is a medicinal product which is either:

- a gene therapy medicinal product
- a somatic cell therapy medicinal product (allogenic, autologous, or xenogenic)
- a tissue engineered product

They all involve a degree of manipulation in-vitro

Why do we need it?

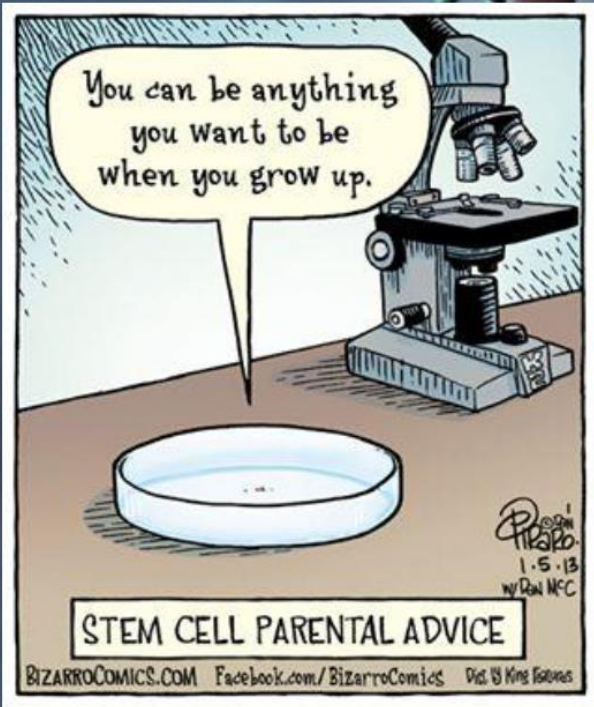
(Lack of donor organs used to be the reason)
Allopathy cannot “cure” 21st century diseases
like :

- Ageing & degeneration
- Auto immune diseases
- Cancer
- Obesity
- Or genetic disorders

(what do they have in common?, what diseases can
be cured with allopathy?)

ATMP

The ~~Beauty~~ and the ~~Beast~~ Genes Cells



ATMPs:

- Gene therapy medicinal products
- Somatic cell therapy medicinal products
- Tissue engineered products



Credit: Christoph Bock/Max Planck Institute for Informatics

Pinterest.com

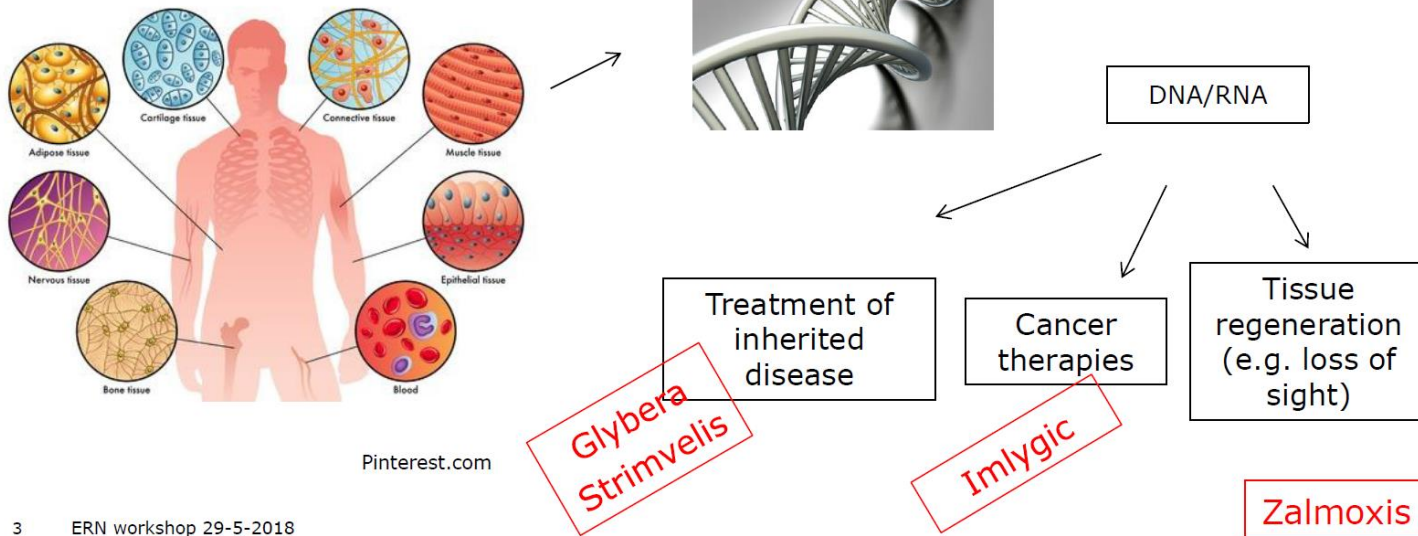
DA: http://www.ema.europa.eu/docs/en_GB/document_library/Presentation/2018/06/WC500250782.pdf

GTMP



EUROPEAN MEDICINES AGENCY

Gene therapy medicinal products

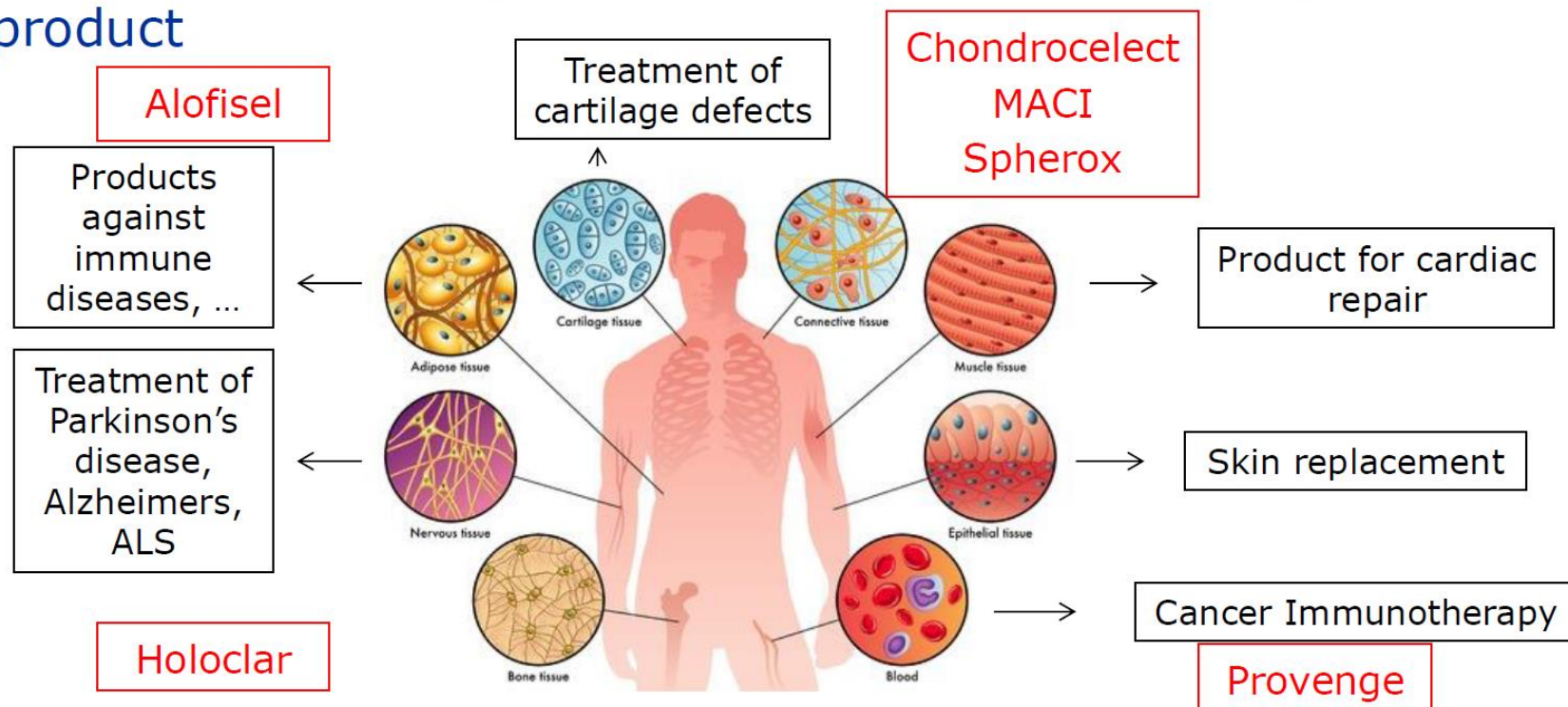


sCTMP



EUROPEAN MEDICINES AGENCY

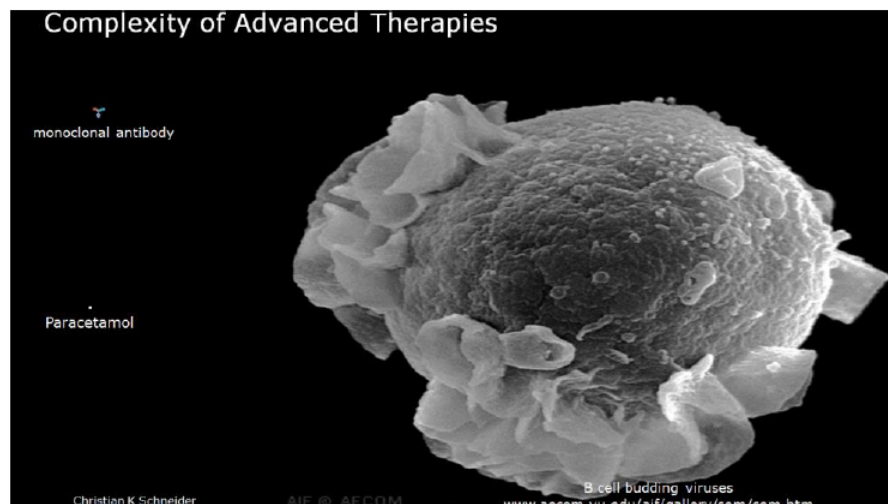
Somatic cell therapy medicinal product – tissue engineered product





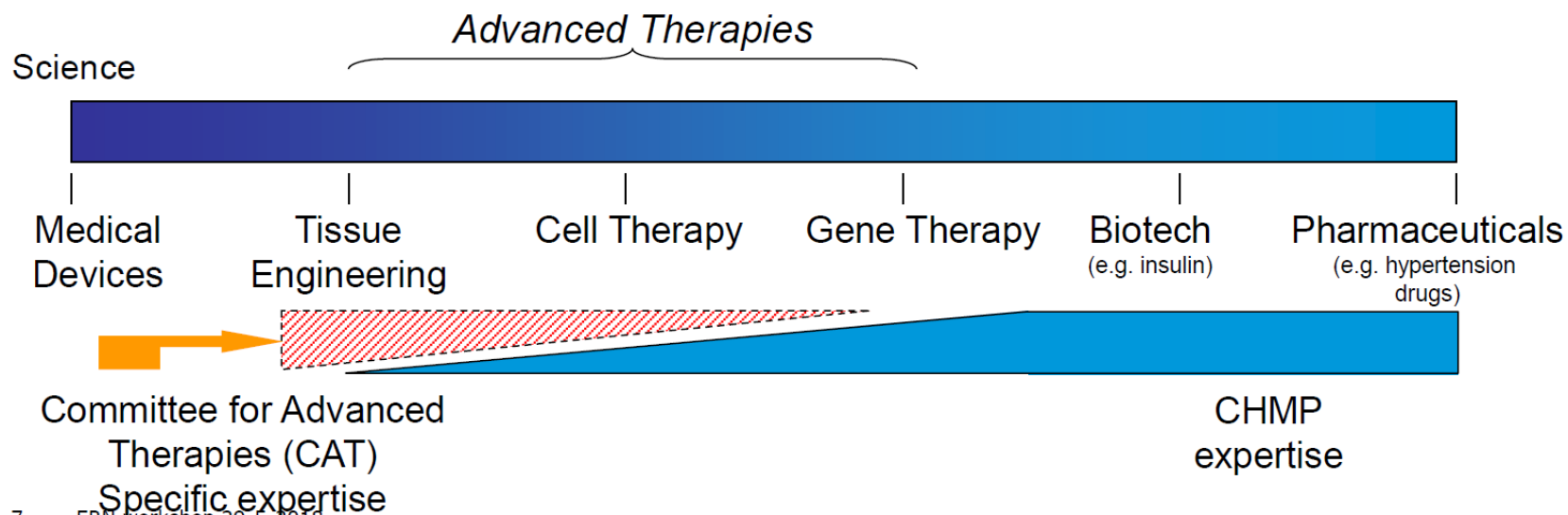
ATMPs are ...

- Medicinal products based on cells or genes
- Very different from medicines based on chemical entities or biological / biotechnological origin
- But same requirement for testing / controlling each batch
 - Impact on cost of manufacture of the ATMPs
 - Very small batch size (autologous CBMP: batch size = 1)





Legislation





ATMPs and the EU legal framework – Lex specialis

10.12.2007

EN

Official Journal of the European Union

L 324/121

REGULATION (EC) No 1394/2007 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 13 November 2007

**on advanced therapy medicinal products and amending Directive 2001/83/EC
and Regulation (EC) No 726/2004**

(Text with EEA relevance)

ATMPs in Europe (May 2018)



EUROPEAN MEDICINES AGENCY

over 500 clinical trials using ATMPs in EU

298 ATMP classifications

293 scientific advice requests

20 MAAs reviewed /
Under review



10 ATMPs approved

Market

3 withdrawn
1 Suspended

6
licensed
ATMPs



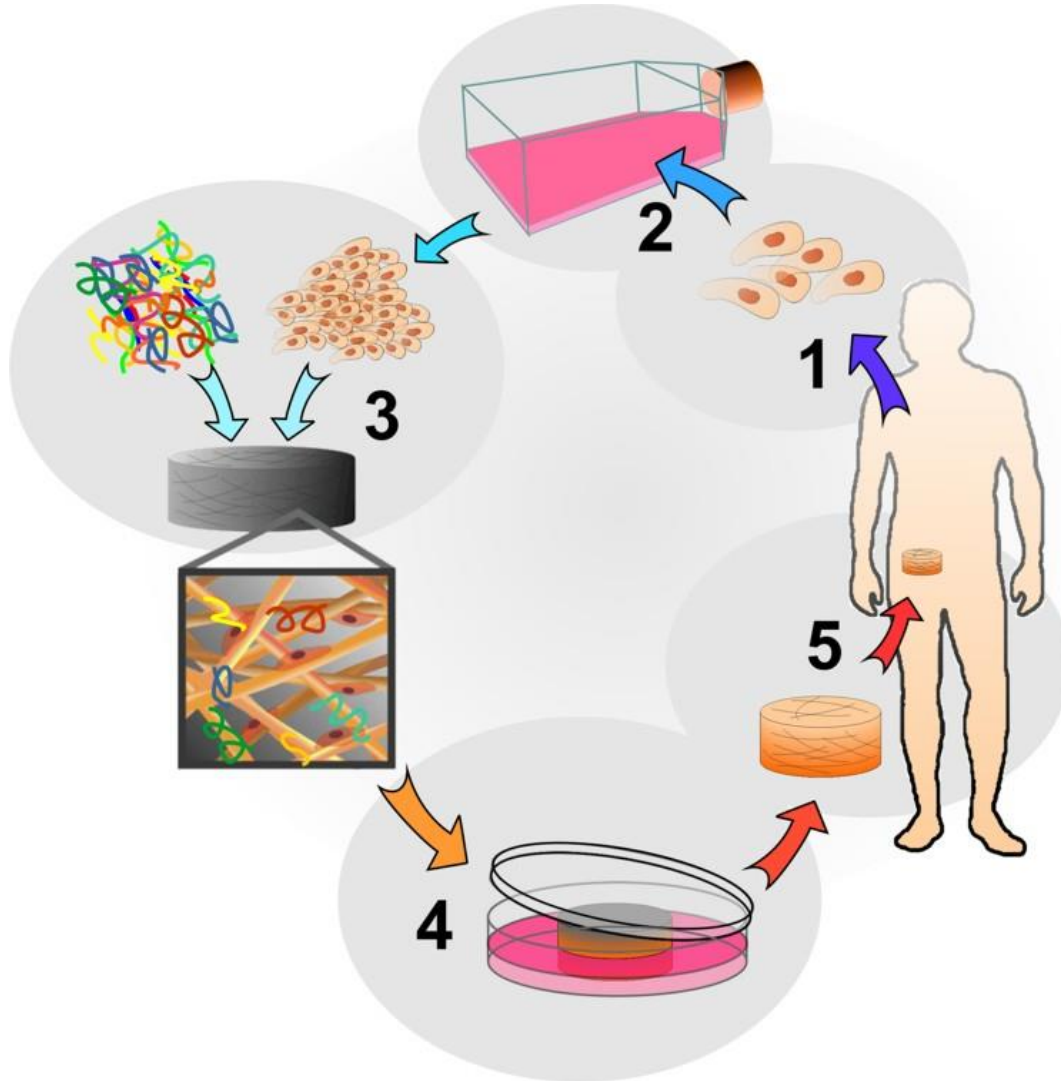


European Commission-DG Health and Food Safety and European Medicines Agency Action Plan on ATMPs

The term “advanced therapy medicinal products” (“ATMPs”) is used to designate gene therapies, somatic cell therapies and tissue engineered products.

In the EU, these products are governed by Regulation 1394/2007 on advanced therapy medicinal products (“ATMP Regulation”). The cornerstone of the Regulation is that a marketing authorisation must be obtained prior to the marketing of ATMPs. The evaluation of these products is led by a specialised committee within the European

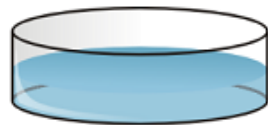
What is Tissue engineering?



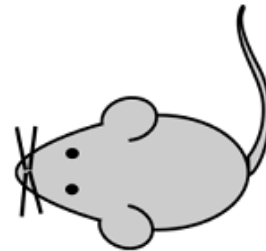
The **old** cells on a scaffold approach

What is an *in vitro* model?

In-vitro models are replicates of the structure and function of biological tissues which allow the modelling and predicting physiological responses to a variety of stimuli.



In Vitro



In Vivo

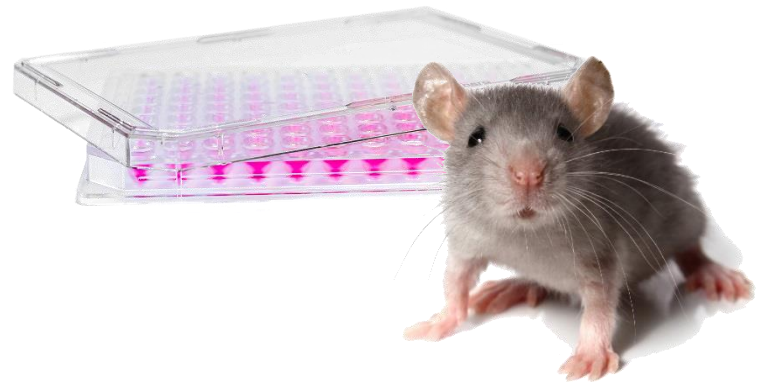
in vitro vs *in vivo*



- *in vivo* experiments are more labour-intensive, expensive and time-consuming than *in vitro* studies.
- there are ethical reasons for limiting the number of test animals to a necessary minimum. The Reduce, Refine and Replace animal experiments (3R) initiative
- human tissue models are thought to have higher predictive power than animal models, because different species respond differently to treatments or compounds

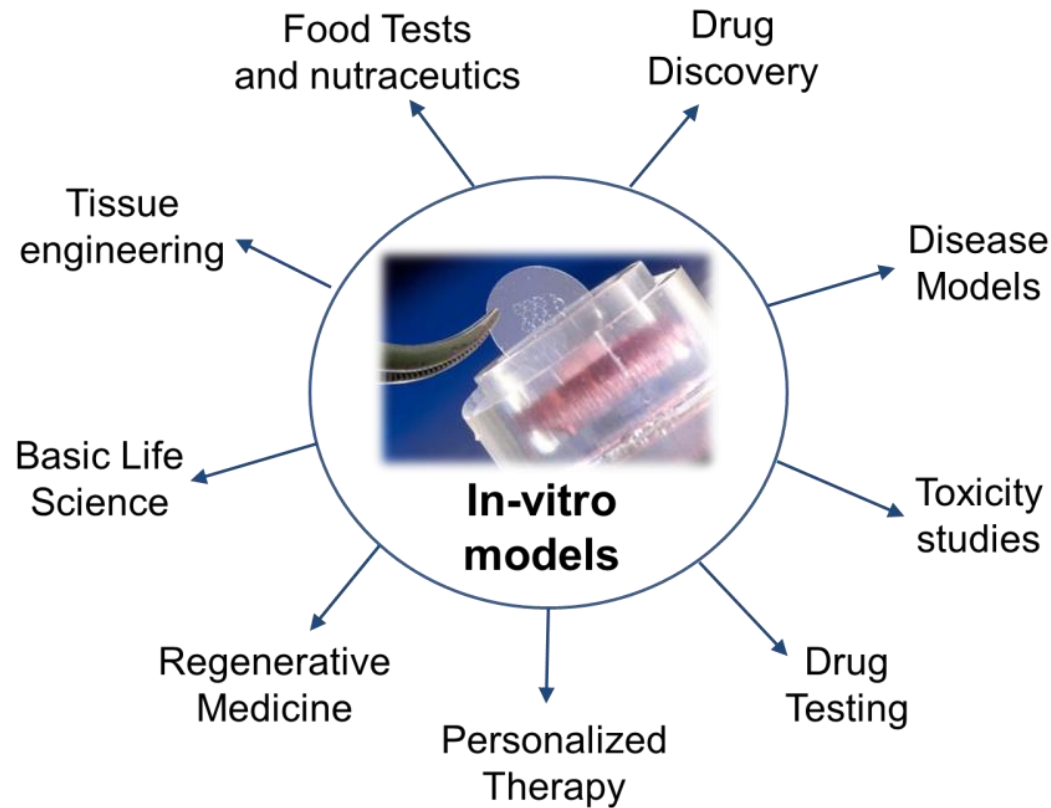


- many of the currently used *in vitro* models lack predictive power, in most cases due to the lack of critical molecular and physical cues in the cell/tissue environment.



in silico

In-vitro models: Applications



DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 22 September 2010

on the protection of animals used for scientific purposes

Article 1

Subject matter and scope

1. This Directive establishes measures for the protection of animals used for scientific or educational purposes.

To that end, it lays down rules on the following:

- a) the **replacement** and **reduction** of the use of animals in procedures and the **refinement** of the breeding, accommodation, care and use of animals in procedures;
- b) the origin, breeding, marking, care and accommodation and killing of animals;
- c) the evaluation and authorisation of projects involving the use of animals in procedures;
- d) the evaluation and authorisation of projects involving the use of animals in procedures

DECRETO LEGISLATIVO 4 marzo 2014, n. 26

Attuazione della direttiva 2010/63/UE sulla protezione degli animali utilizzati a fini scientifici. (14600036)

(GU n.61 del 14-3-2014)

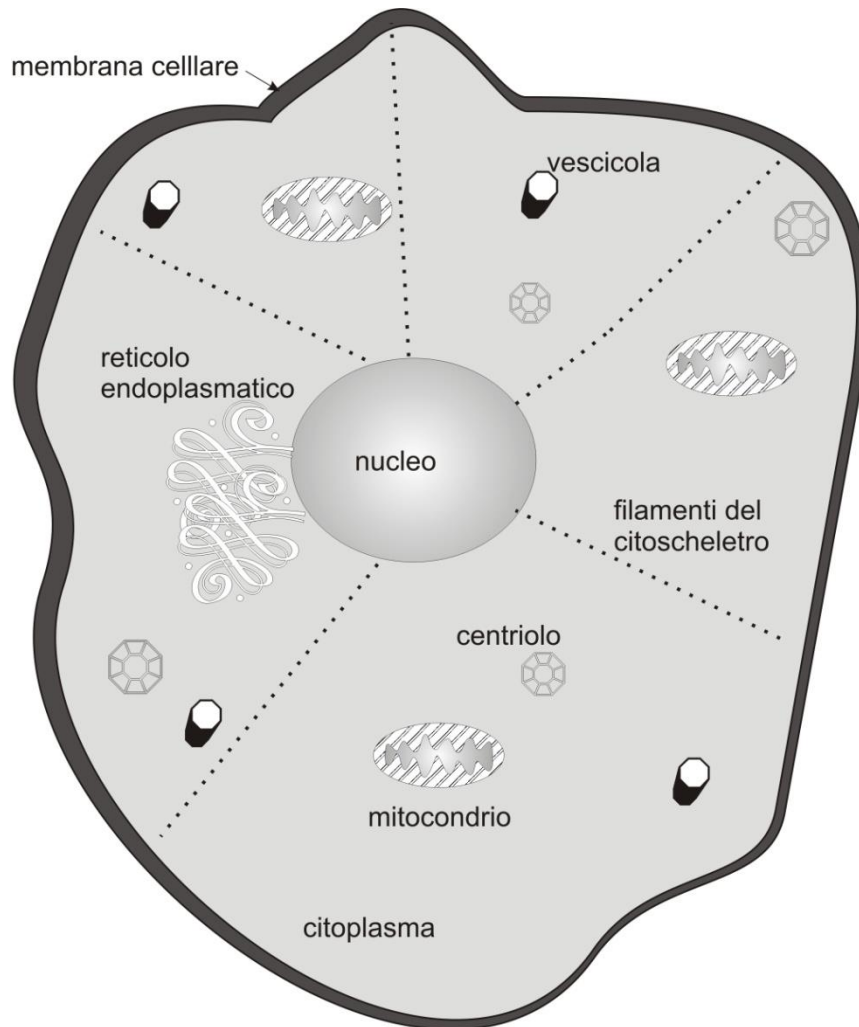
The 3Rs (Russel & Burch, 1959)



Centro 3R

Centro Interuniversitario per la Promozione dei Principi delle 3R nella Didattica e nella Ricerca

La cellula



Dovete sapere le funzioni
dei componenti
citoplasmici

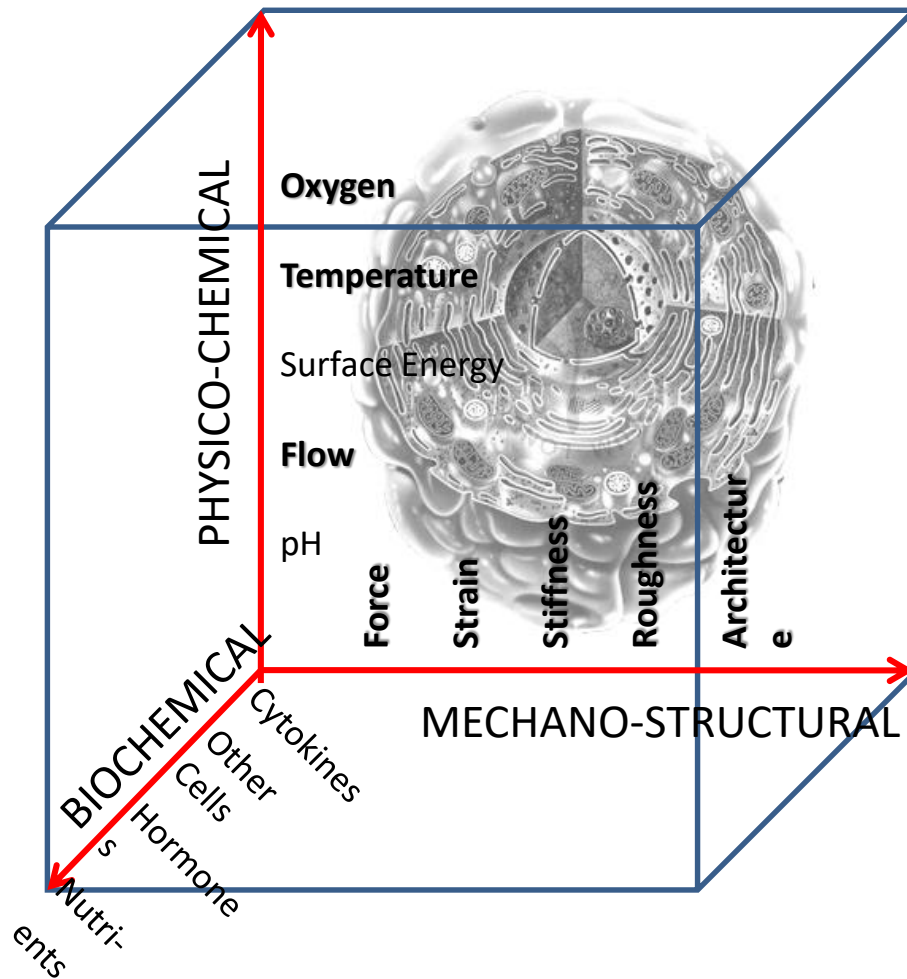
Cellule matrice dipendenti
e cellule "libere"

La Matrice Extra Cellulare

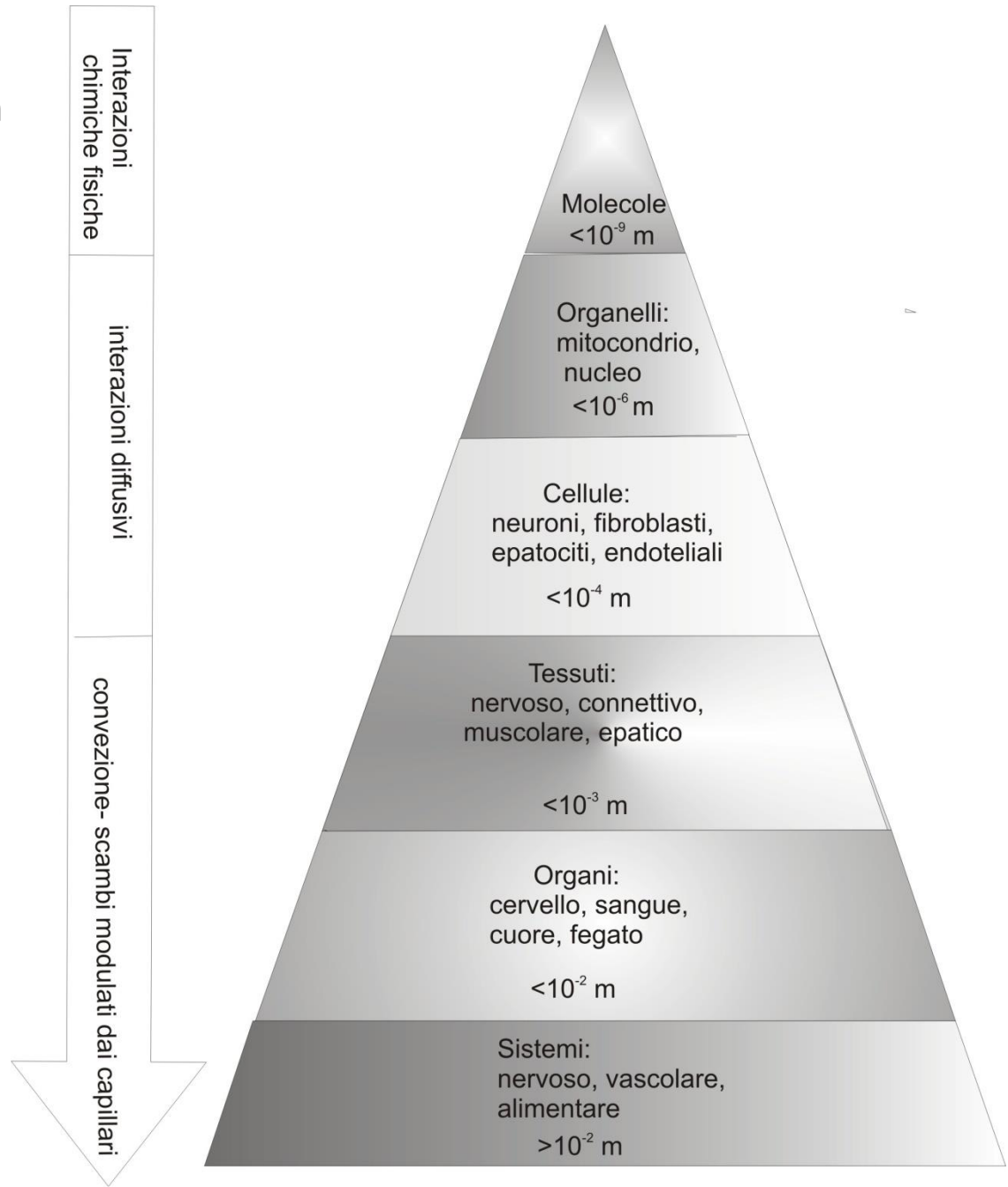
(vedere anche la roba di biomeccanica sul ECM)

Matrice Extra Cellulare	
Componente	Funzione
Acqua	E' il mezzo di trasporto, ed è la componente più importante degli organismi viventi. Rende inoltre incompressibile L'ECM,.
Sali Minerali	Mantengono un sistema tamponato
Elastina	Proteina strutturale
Fibronettina, laminina ..	Proteine adesive specializzate, spesso glicosilate
Glicosamminoglicani	Disaccaridi (ad esempio:acido ialuronico, eparina, eparan solfato) che formano un complesso con le proteine per formare i proteoglicani
Proteoglicani	Complessi zuccheri-proteine che formano un reticolo macromolecolare o gel idratato, figura 4
Collagene	Proteina strutturale e ligando adesivo

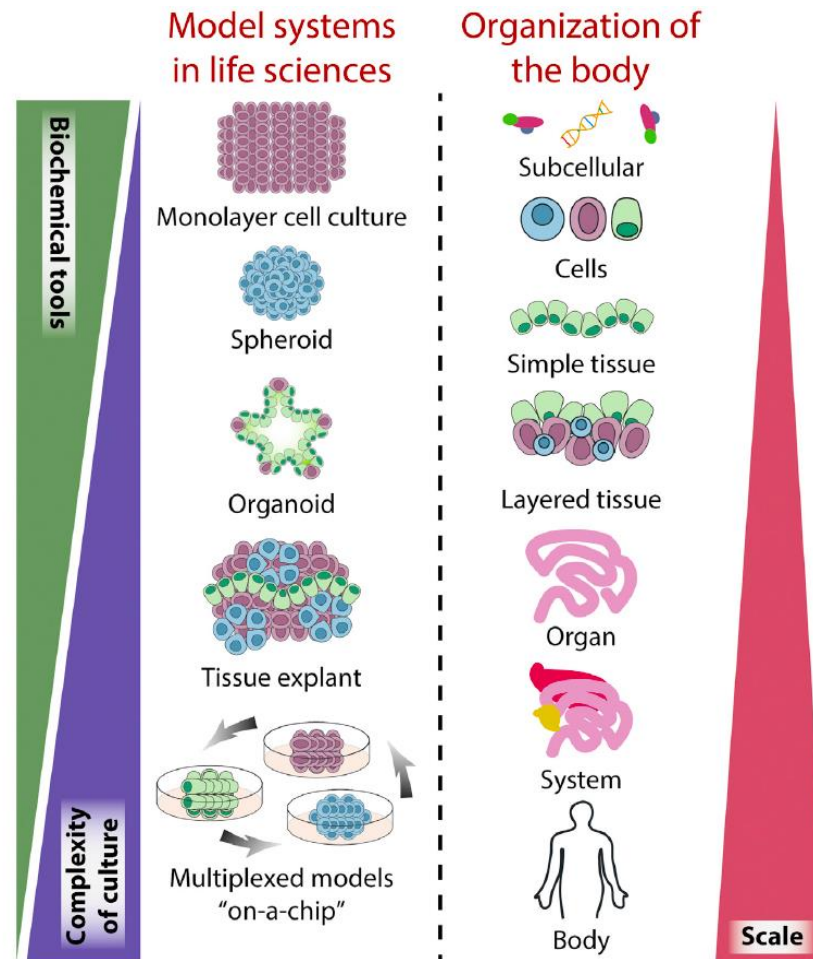
monitoring/sensing/control is essential



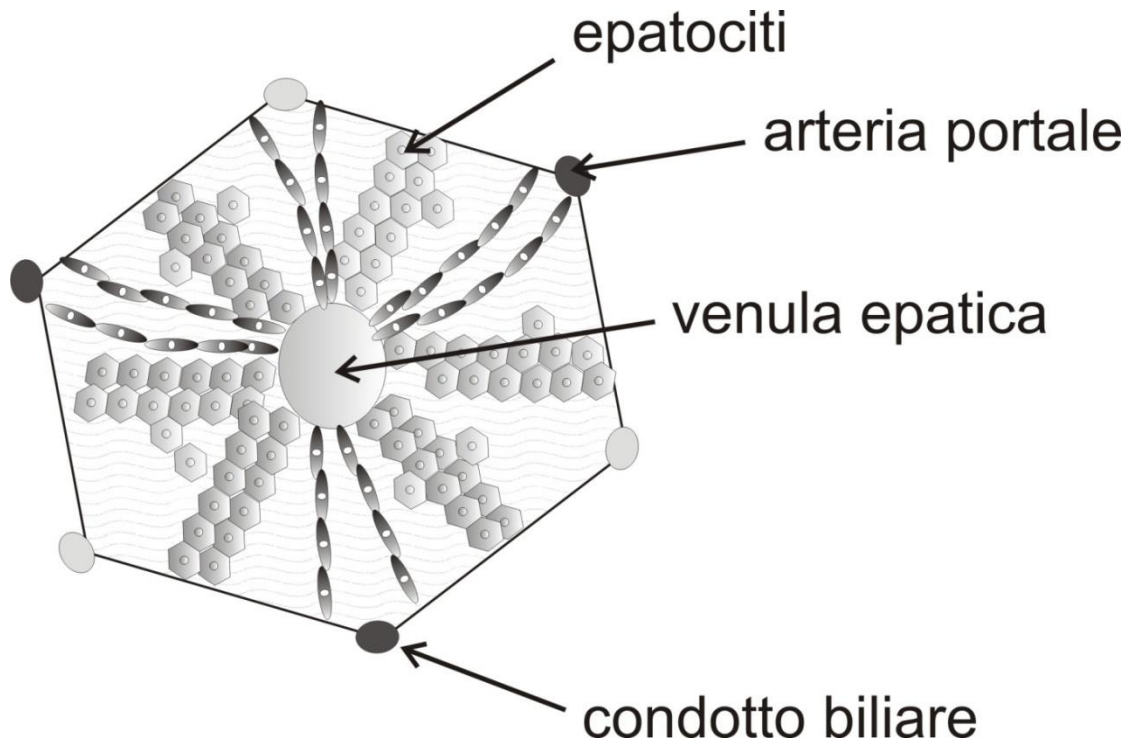
Hierarchical organisation



Biomimetic tissue models

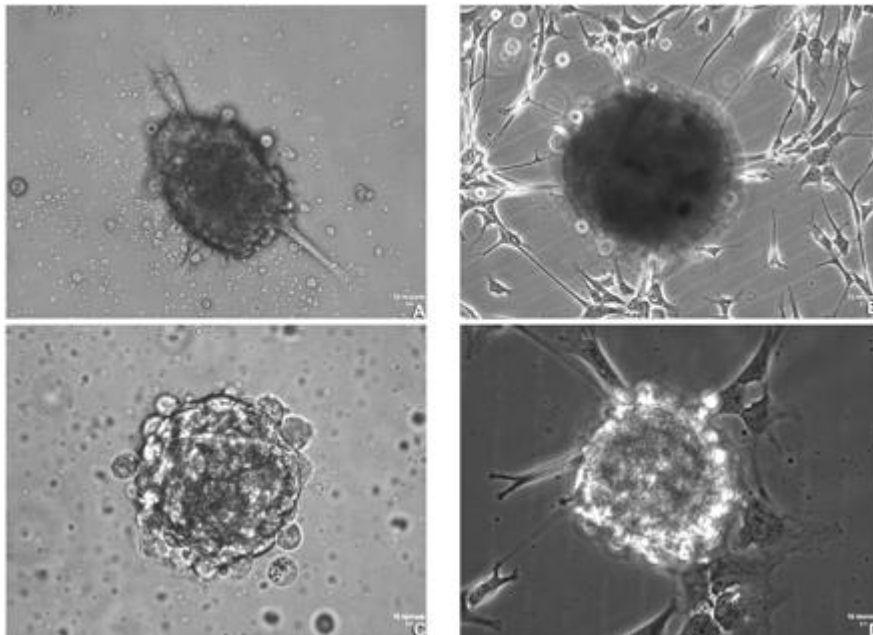


Functional unit: collection of functional (parenchymal) and support (stromal or non-parenchymal) cells which do not require a capillary network. Is equivalent to a cube of 400 micron sides. In vitro these units are usually referred to as **ORGANOIDS**



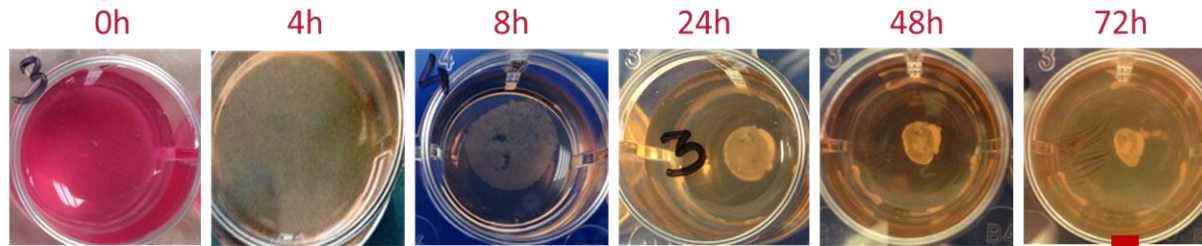
Functional unit

- Each organ is a network of the parallel functional units, composed of groups of functional cells or parenchymal supported by stromal cells, each unit has dimensions of a few hundreds of microns, and responds with characteristic times in the order of minutes. The micro-functional domains are repeated both in morphology and function.



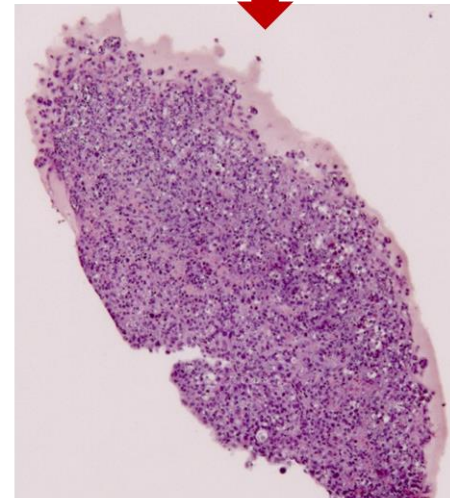
Cardiospheres and organoids are a good example

Hepatic organoids



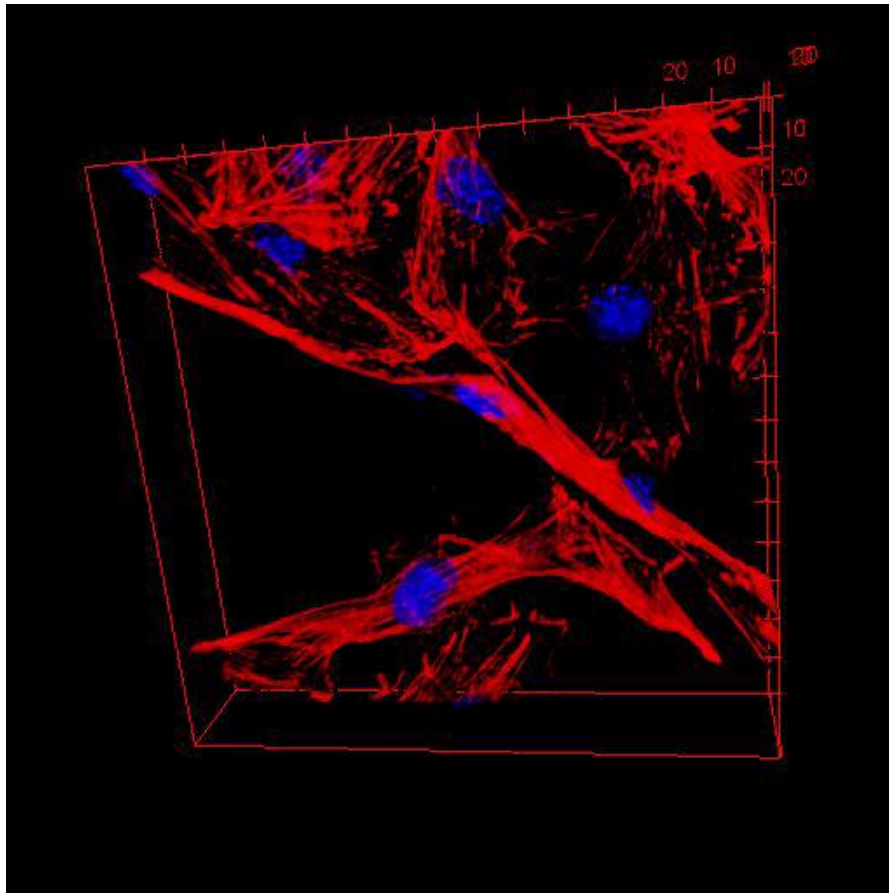
Combination of:
differentiated, human upcyte® hepatocytes
+ upcyte® LSECs
+ upcyte® MSCs

HE-stain of a
„first try“ liver
bud (72h)



Numbers

- The typical cell– diameter 10-20 μm



How many?
How many in an organ?
How many are therapeutic?

RESEARCH PAPER

An estimation of the number of cells in the human body

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Abstract

Background: All living organisms are made of individual and identifiable cells, whose number, together with their size and type, ultimately defines the structure and functions of an organism. While the total cell number of lower organisms is often known, it has not yet been defined in higher organisms. In particular, the reported total cell number of a human being ranges between 10^{12} and 10^{16} and it is widely mentioned without a proper reference.

Aim: To study and discuss the theoretical issue of the total number of cells that compose the standard human adult organism.

Subjects and methods: A systematic calculation of the total cell number of the whole human body and of the single organs was carried out using bibliographical and/or mathematical approaches.

Results: A current estimation of human total cell number calculated for a variety of organs and cell types is presented. These partial data correspond to a total number of 3.72×10^{13} .

Conclusions: Knowing the total cell number of the human body as well as of individual organs is important from a cultural, biological, medical and comparative modelling point of view. The presented cell count could be a starting point for a common effort to complete the total calculation.

Keywords

Cell size, human cell number, organ, total cell count, theoretical issue

History

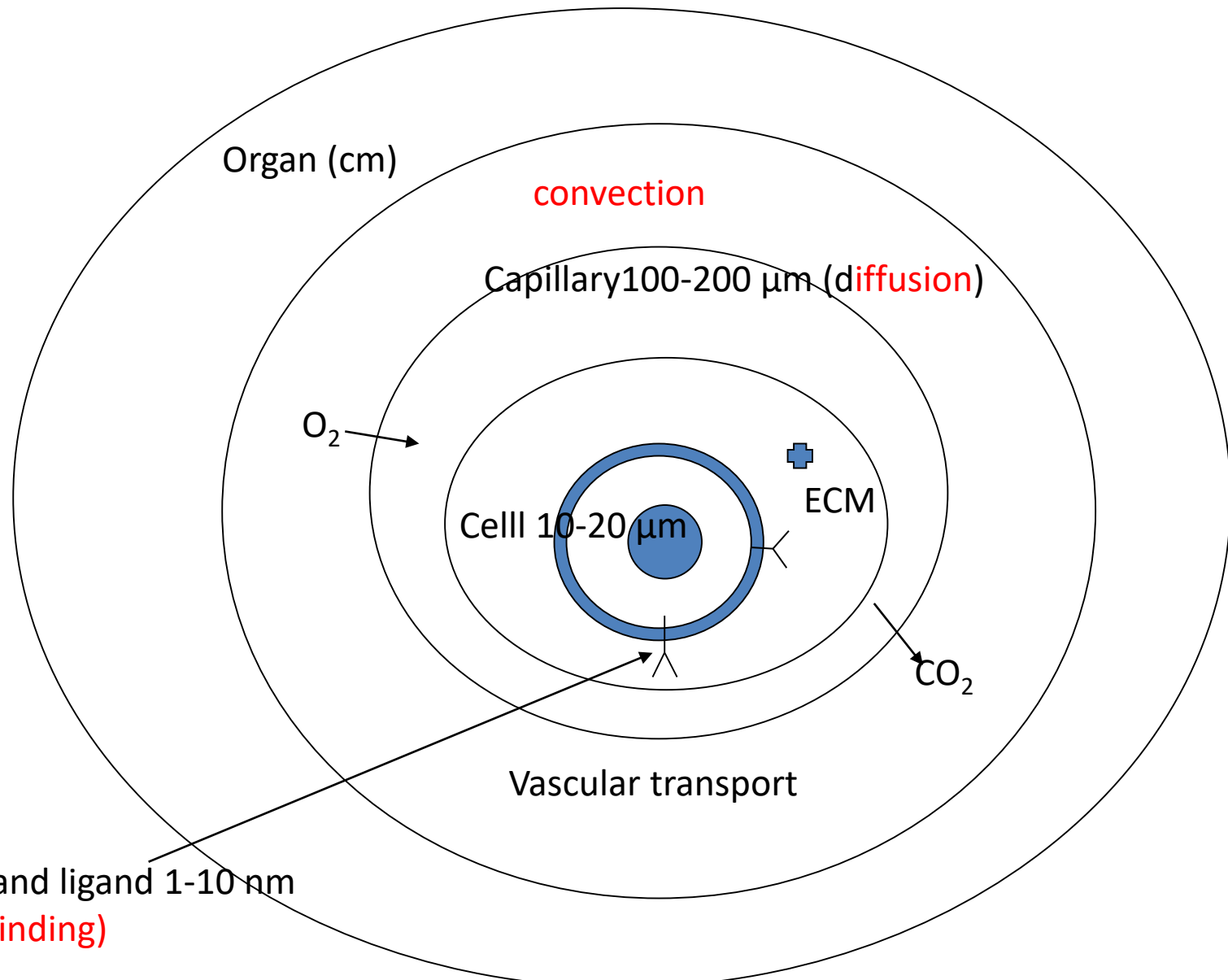
Received 26 September 2012

Revised 19 March 2013

Accepted 9 May 2013

Published online 5 July 2013

Characteristic distance 100-500 μm



Quali sono i processi cellulari fondamentali?

Genes hold the gun
Environment pulls the trigger

OMICS
Genome
Phenome
Epigenome
Connectome
secretome

Quali invece sono specifici a cellule specifiche?

Fenotipo – caratteristiche fisiche e biochimiche del organismo

Genotipo- caratteristiche del DNA nucleare

Epigenotipo- alterazione del espressione genica da fattori ambientali

Le funzioni cellulari sono diverse da cellula a cellula e da tessuto a tessuto, e definiscono il **fenotipo** cellulare. Però alcuni processi sono comuni a tutte le cellule. I processi cellulari più noti sono:

- Proliferaazione o crescita
- Migrazione
- Differenzazione
- Morte (apoptosi, necrosi)
- Metabolismo, respirazione
- Adesione
- Espressione proteica

Define: phenotype, genotype, epigenotype

Definitions

- **Epigenetics** is the study, in the field of genetics, of cellular and physiological phenotypic trait variations that are caused by external or environmental factors that switch genes on and off and affect how cells read genes instead of being caused by changes in the DNA sequence. The consensus definition now requires a trait to be heritable for it to be considered epigenetic. Epigenetics is the reason why a skin cell looks different from a brain cell or a muscle cell. All three cells contain the same DNA, but their genes are expressed differently (turned "on" or "off"), which creates the different cell types.

Genes load the gun
Environment pulls the trigger



OMICS
Genome
Phenome
Epigenome
Connectome
Secretome
Organome
Inflammatome

Fenotipo – caratteristiche fisiche e biochimiche del organismo

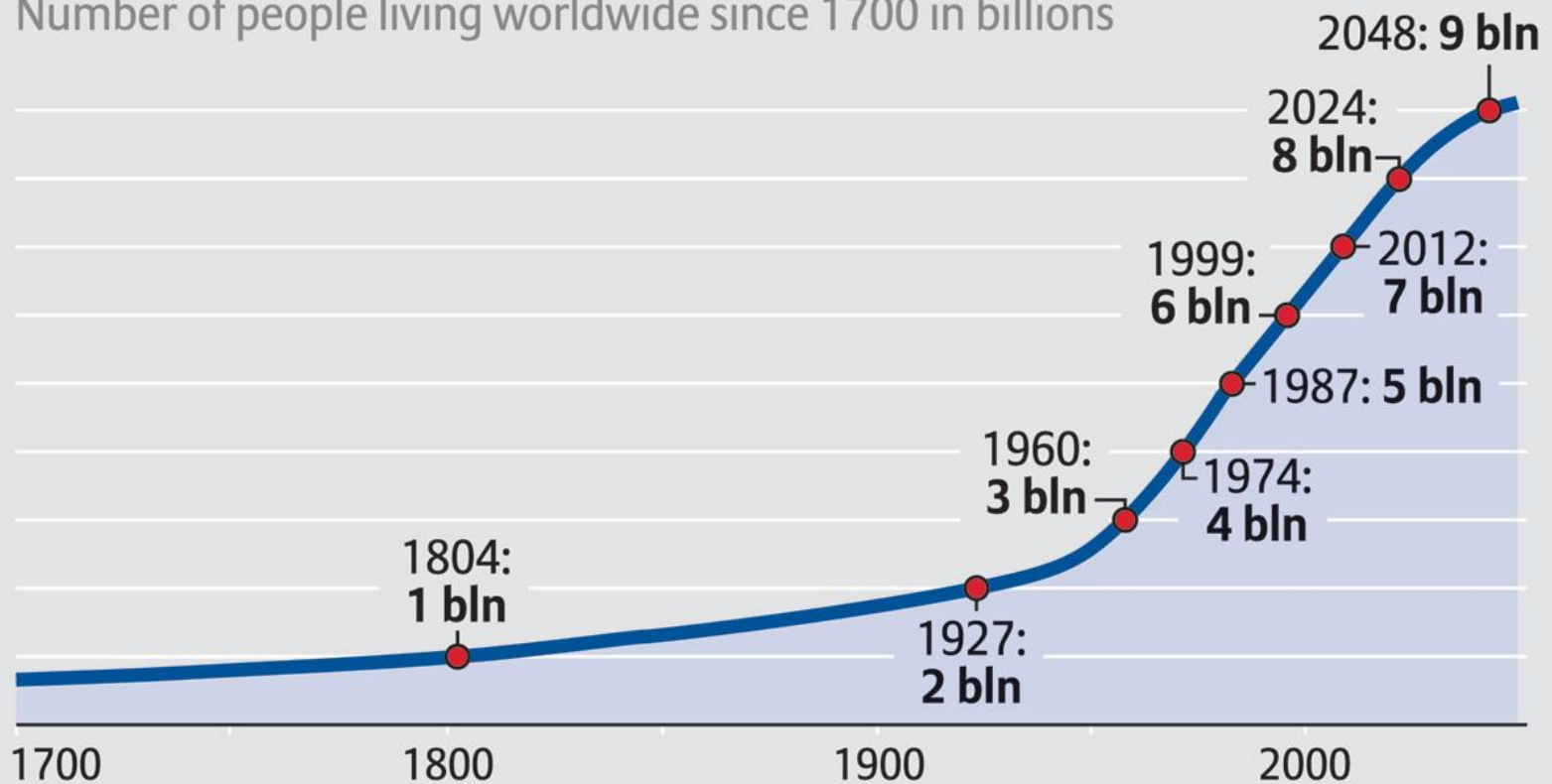
Genotipo- caratteristiche del DNA nucleare

Epigenotipo- alterazione del espressione genica da fattori ambientali
(DNA methylation)

POPULATION OF THE EARTH



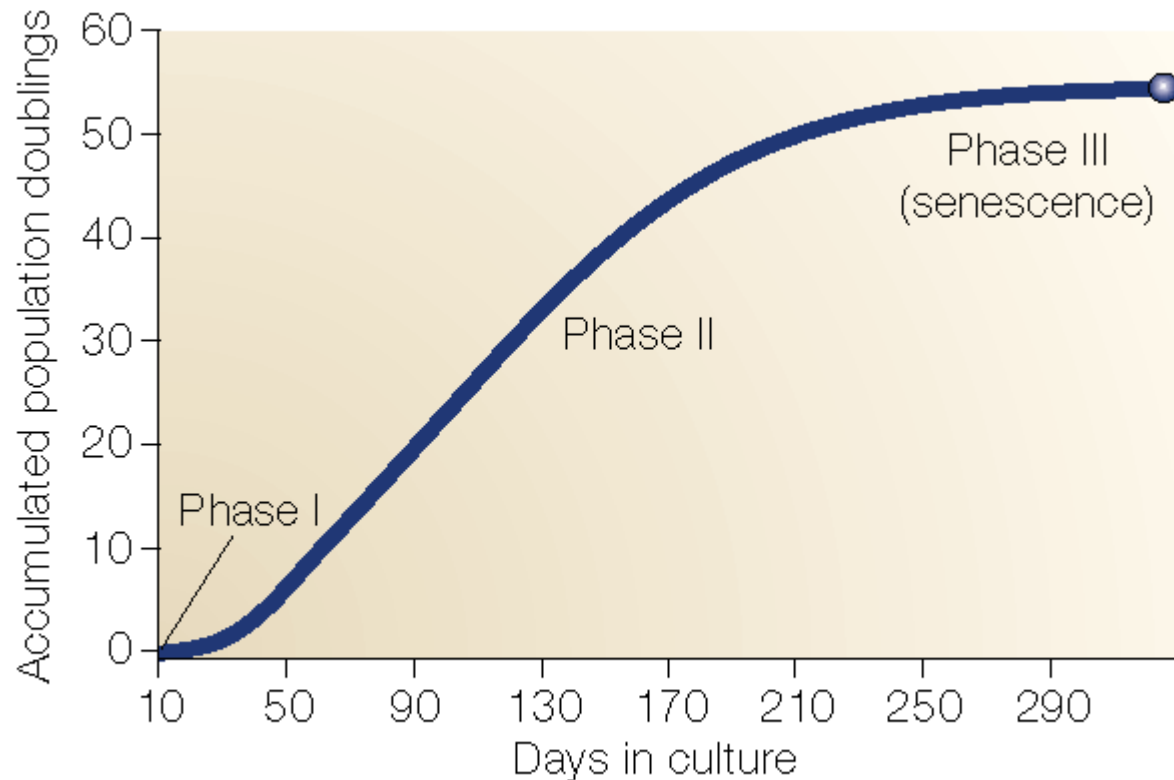
Number of people living worldwide since 1700 in billions



Source: United Nations World Population Prospects, Deutsche Stiftung Weltbevölkerung

For further information please visit: www.knowledge.allianz.com

Crescita' cellulare



Hayflick, L.. The limited in vitro lifetime of human diploid cell strains. 1965

Rate of cell proliferation is
proportional to cell number

$$\frac{dN}{dt} \propto N$$

$$\frac{dN}{N} = k dt$$

$$N = N_o e^{kt}$$

$$2N = N_o e^{kt_d}$$

$$t_d = \frac{\ln 2}{k}$$

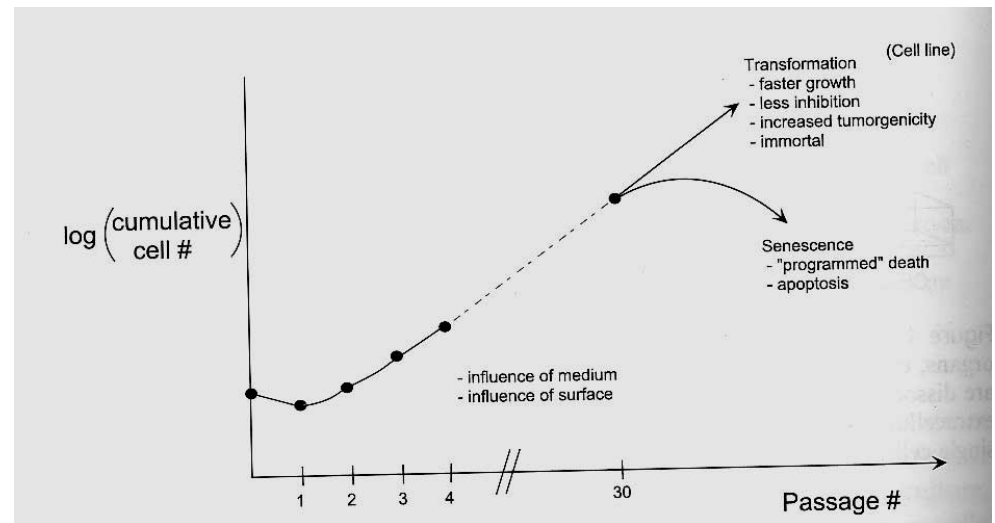
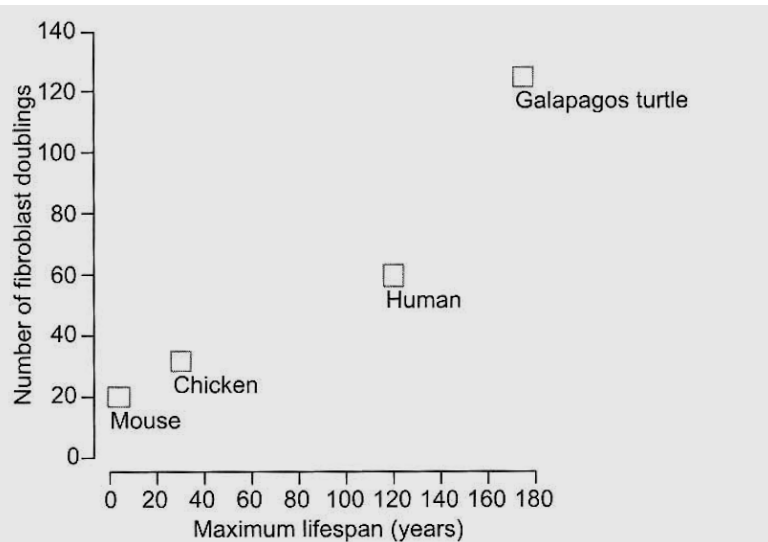
N= cell population

N_o= initial
population @t=0

t_d =population
doubling time

Cell growth: Hayflick limit and population doublings

The [Hayflick limit](#) is the theoretical limit to the number of times a cell may divide until the telomere becomes so short that division is inhibited and the cell enters senescence.



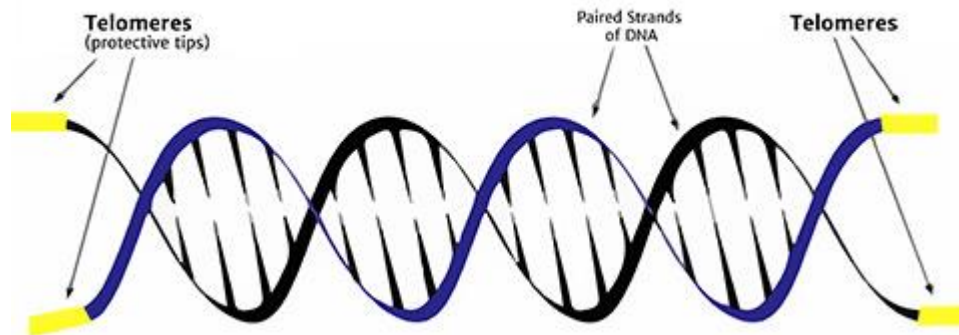
The Immortal Life of Henrietta Lacks

Telomere

A region of repetitive nucleotide sequences at each end of a chromosome, which protects the end of the chromosome from deterioration or from fusion with neighboring chromosomes.. Telomeres get shorter each time a cell copies itself, but the important DNA stays intact.

Eventually, telomeres get too short to do their job, causing our cells to age and stop functioning properly. Therefore, telomeres act as the aging clock in every cell. Telomeres are shortened as we age, but can also be shortened by stress, smoking, obesity, lack of exercise and a poor diet .

Telomere shortening is involved in all aspects of the aging process on a cellular level. Telomere length represents our biological age as opposed to our chronological age.



Esercizi e domande

- Cose' un ATMP?
- Quale' la differenza regolatorio tra un ATMP e' un farmaco?
- Questa e' una lista di ATMP approvati in Europa. Ricercare i prodotti per completare la tabella.

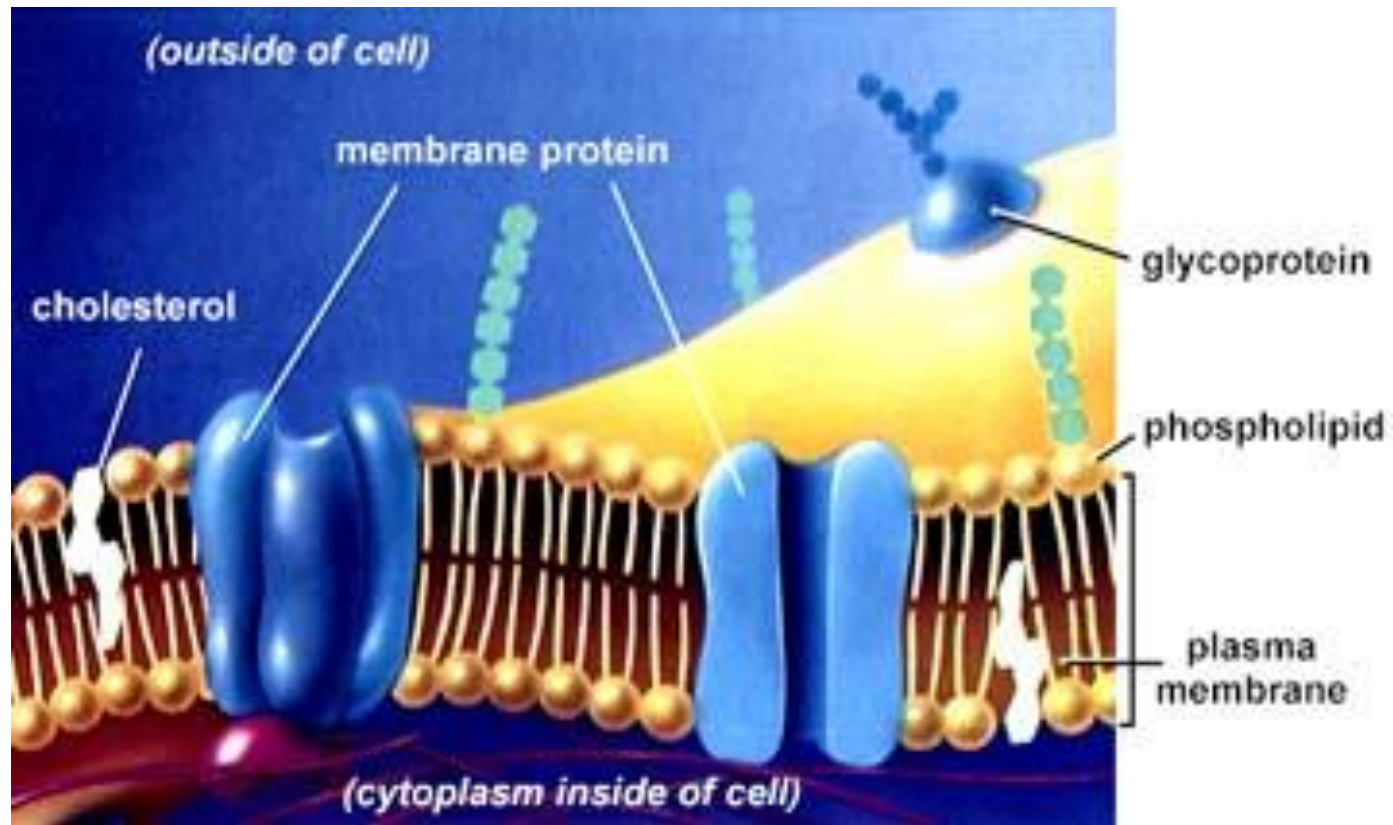
ATMPS

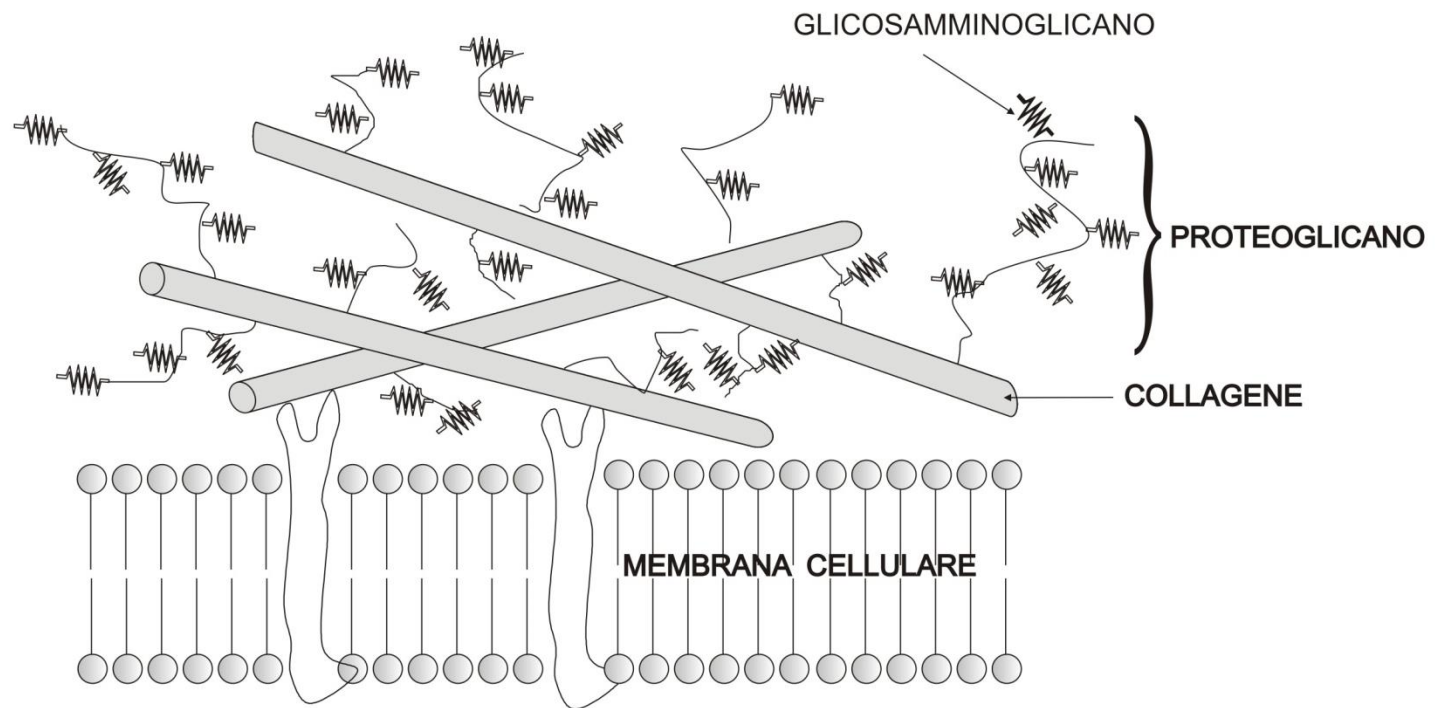
	azienda	malattia	cose	anno	stato	costo
Alofisel	Tigenix	Fistuli anali morbo Crohn	Cellule mesencimali	22-3- 2018	In corso	54K £
Spherox	CO.DON	Cartilagine ginocchi	Sferoidi condrociti autologhe	2017	In corso	
Zalmoxis	Moimed Spa	Leucemia e post trapianto	Cellule staminali allogeniche	2016	Sul mercato ma viene seguito	149K€+iva/tra ttamento
Strimvelis	GSK	ADA-SCID	Trasfezione cellule staminali autologhe	2016	In corso	595K sterline/tratt, per 5 anni 2.5M £

	azienda	malattia	cose	anno	stato	costo
Imlygic	Amgen	melanoma	Virus modificato	2015	Sul mercato/ viene seguito	65K £
Holocar	Chiesi IT	Cornea ustionata	Cellule corneali	2015	In corso	80K \$
Provenge	Dendron USA	Cancro prostata	Immunocit i autologhi	2013	Ritirato in EU 2015	100K\$
MACI						
Glybera	uniQure	LPLD	Vettore virale con gene LPL sano	2012	Ritrato in 2017	\$1.5M
Chondrocelect	Ti Genix	Cartilagine ginocchio	Cellule autologhe espanse		Ritirato in 2016	24K £

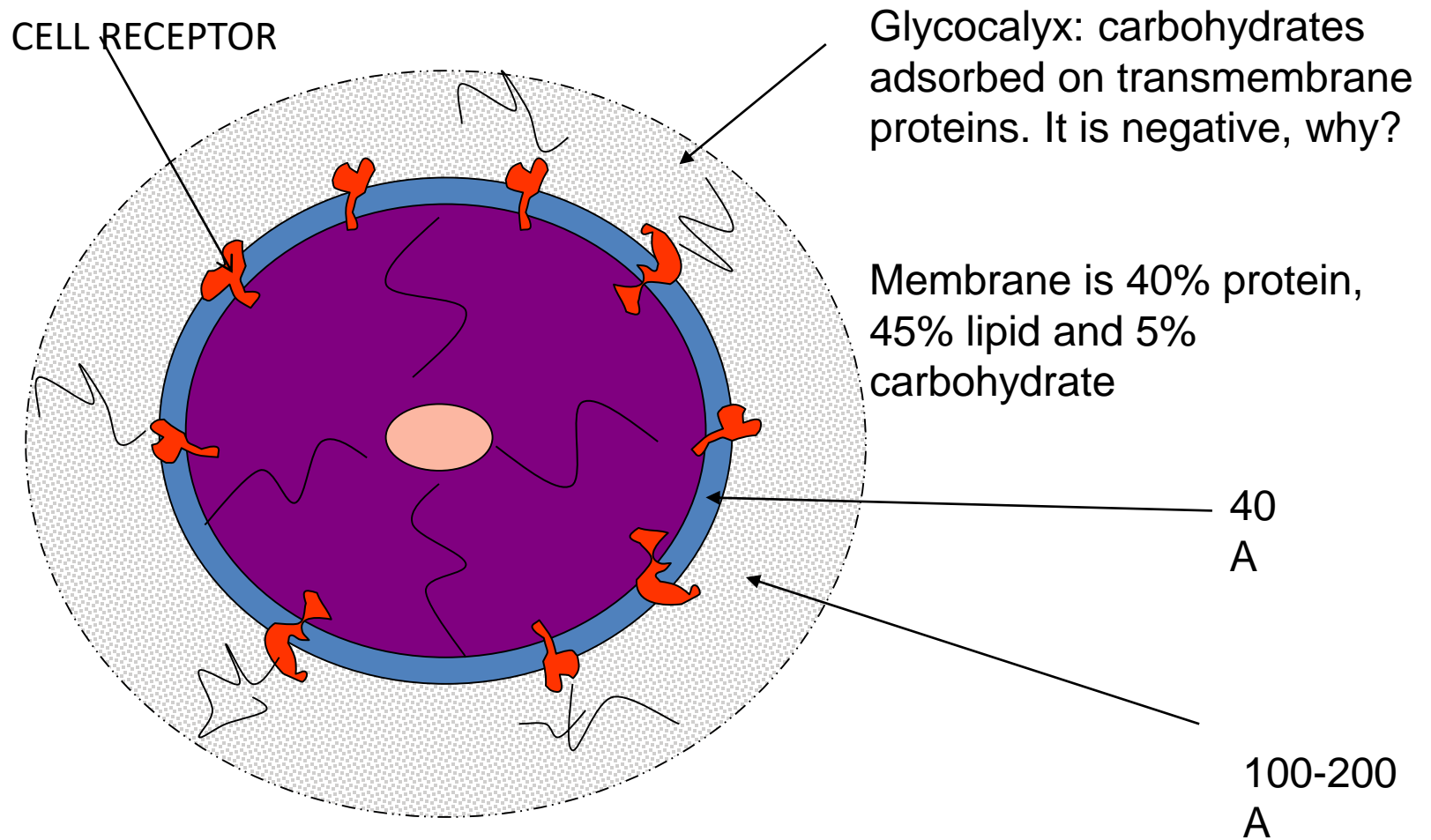
LIGAND BINDING/RECEPTORS/ CELL ADHESION

Libro di Lauffenburger e Linderman

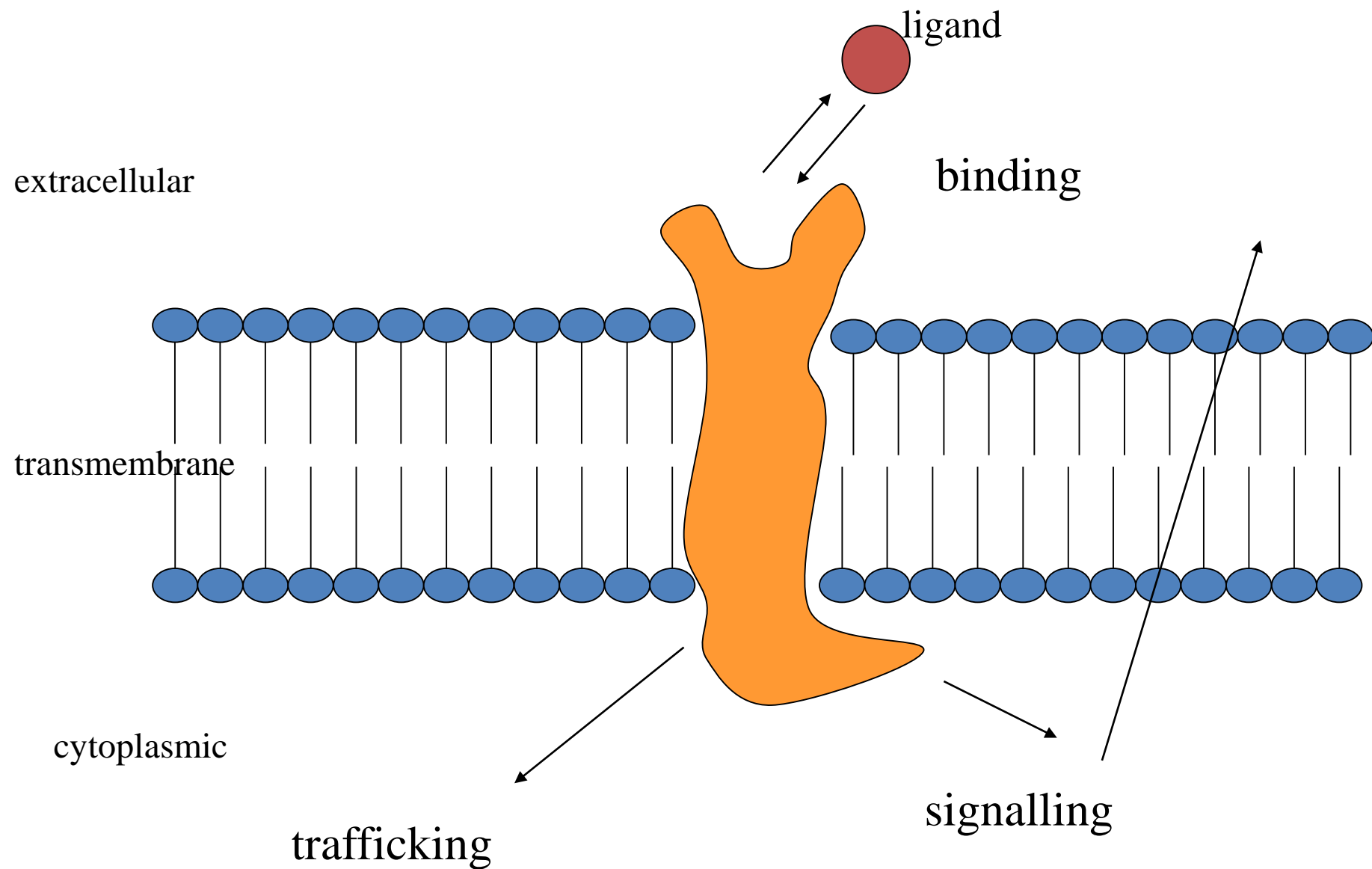




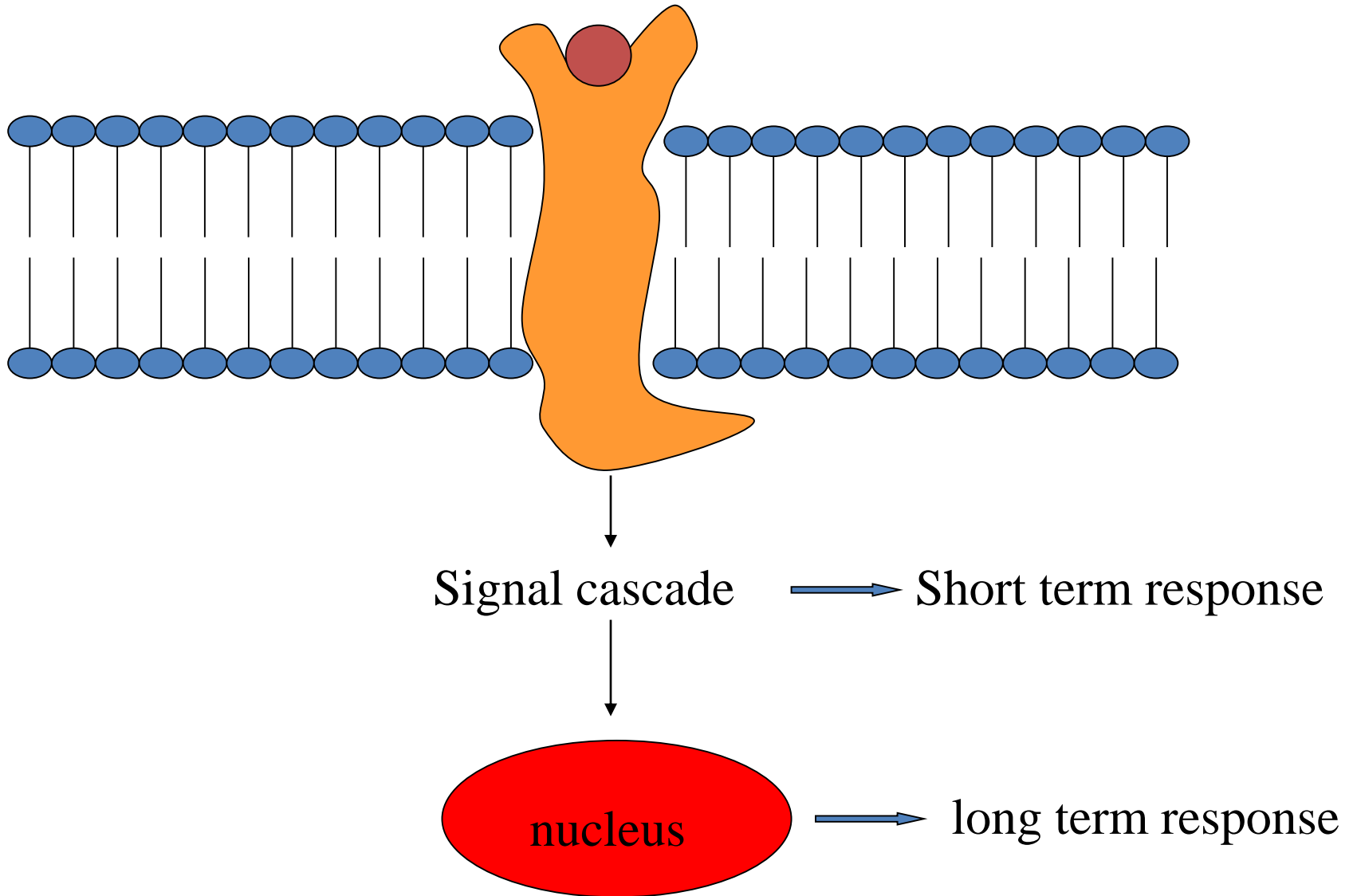
Binding



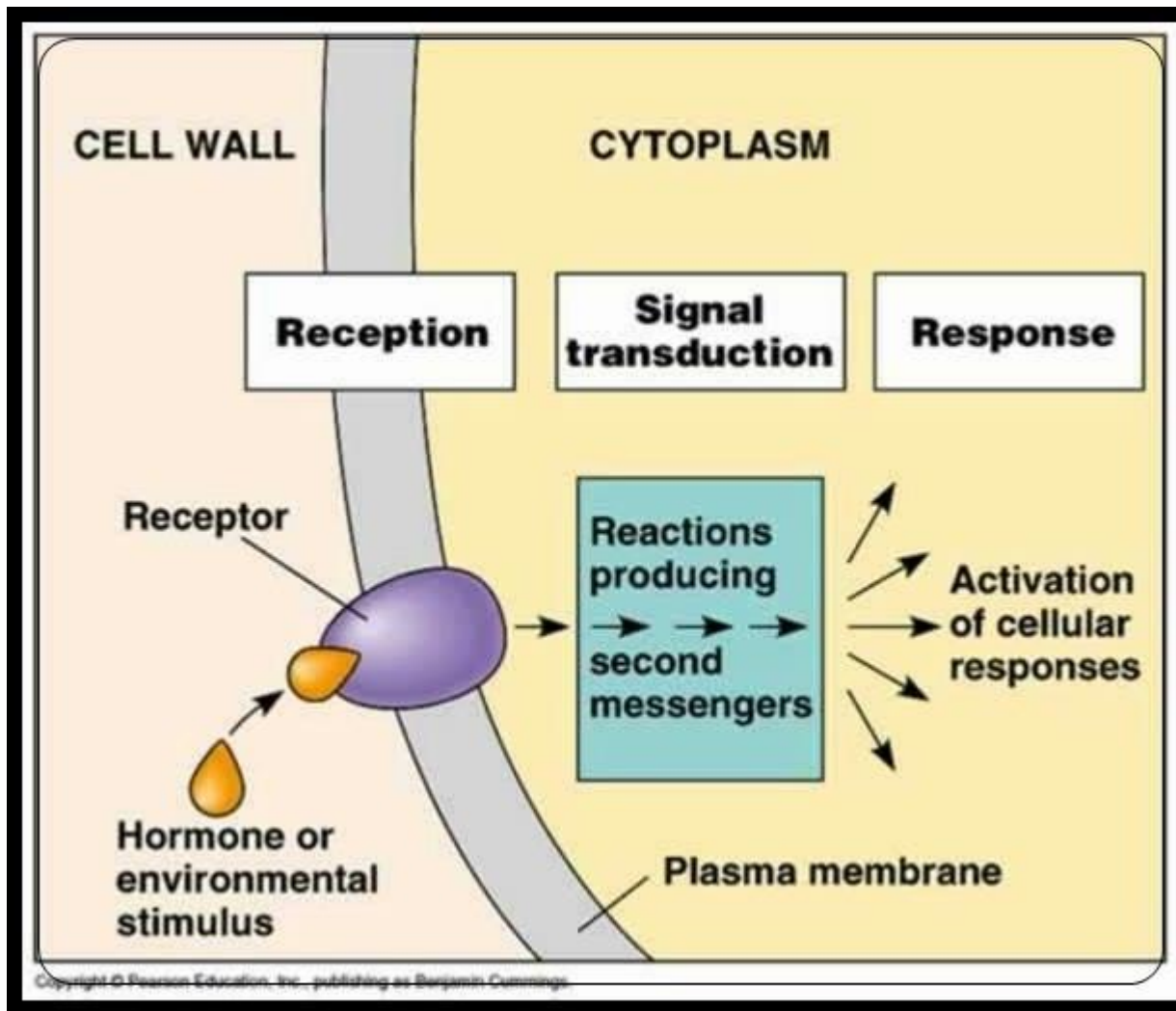
Eukaryotic Cell responses are regulated and controlled by receptor interaction with the environment. So parameters such as growth, death, differentiation, are studied by analysing receptor-ligand binding and the associated trafficking and signalling events.



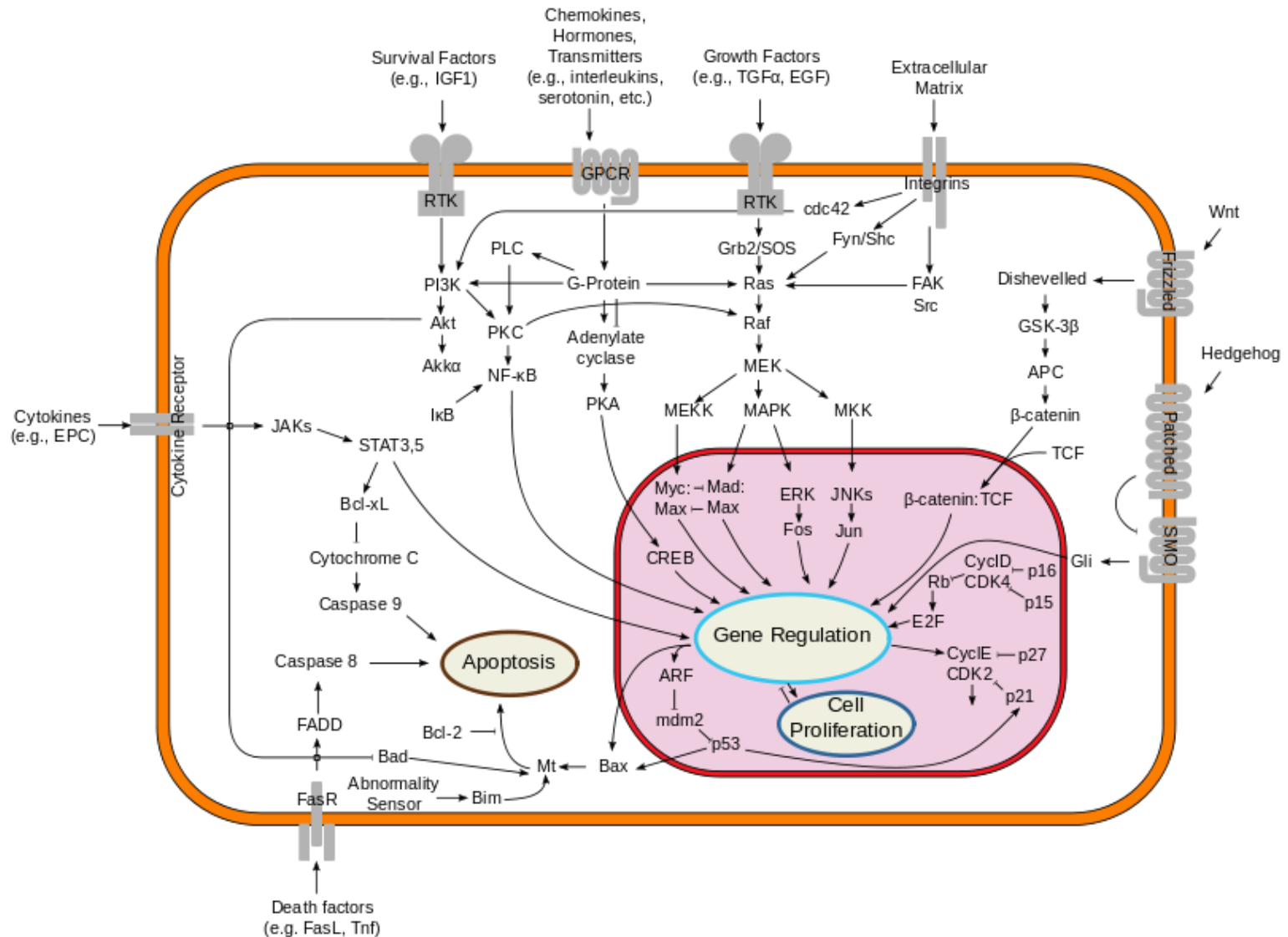
INSIDE OUT- OUTSIDE IN



Signal transduction occurs when an extracellular signaling molecule activates a specific receptor located on the cell surface or inside the cell. In turn, this receptor triggers a biochemical chain of events inside the cell, creating a response. Depending on the cell, the response alters the cell's [metabolism](#), shape, [gene expression](#), or ability to divide. The signal can be amplified at any step. Thus, one signaling molecule can cause many responses.



An example of signal CASCADE and TRANSDUCTION pathways



Receptors: Cell surface receptors (CSR). They interact with the extra cellular environment giving rise to four types of signals:

- Nerve transmission
- Hormone release
- Muscle contraction
- Growth stimulation

There are four types of messenger molecules.

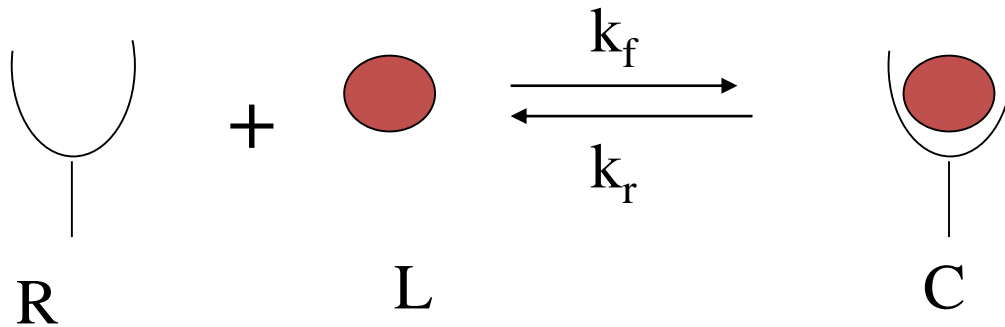
- steroids
- small organic or inorganic molecules
- peptides
- Proteins

The messengers may be

- Endocrine: usually hormones
- autocrine
- paracrine : usually cytokines
- juxtacrine

There are 3 classes of ligand bound receptor signal transduction models

- ion channel receptor (fast ms, low affinity)
- G protein linked receptor (second messenger involved)(medium, mins, med affinity) (GPCR)
- Enzyme (usually Tyrosine kinase i.e. enzyme which adds a phosphate group to proteins at tyrosine residues...ie phosphorylation) linked receptors -Slow and high affinity



We consider a model of receptor-ligand binding in which binding is monovalent and interfering effects are absent. k_f and k_r are the kinetic association and dissociation constants.

R =number of receptors per cell

C =number of complexes per cell

L =conc of ligand in the ECM (moles/liter)

$k_r = t^{-1}$

$k_f = M^{-1}t^{-1}$

N =number of cells per unit volume

ok

A variety of messengers can bind to various tissues.

Various cellular responses may occur, depending on the tissue.

Either positive or negative responses may occur, even in the same tissue, depending on the type of receptor.

The response of a cell to a messenger depends on the number of receptors occupied.

A typical cell may have about 1000-3000 receptors.

Only a small fraction (10%) of the receptors need to be occupied to get a large (50%) response.

Receptors may have a dissociation constant of about 10^{-11} ; this is the concentration of messenger at which they are 50% saturated. Thus very low concentrations of messengers may give a large response.

Receptor	Ligand	Cell	$R_T(\#/cell)$	$K_f(M^{-1}min^{-1})$	$K_r(min^{-1})$	$K_d(M)$	$T_{95\%}(min)$
Fc	Fab	macrophage	7.1e5	3e6	0.023	7.7e-10	650
EGF	EGF	Rat lung	2.5e4	1.8e8	0.12	6.7e-10	12.5
Fibronectin	Fibronectin	fibroblasts	5e5	7e5	0.6	8.6e-7	2.5
Transferrin	Transferrin	hepatocytes	5e4	3e6	0.1	3.3e-8	15

Cell surface receptors CSR

Recettore	Ligando	R_T (numero/cellula)	k_f ($\text{nM}^{-1} \text{min}^{-1}$)	k_r (min^{-1})	K_D (nM)
Trasferrina	Trasferrina (trasportatore ferro negli epatociti)	50000	0.003	0.1	33
EGF	EGF (fattore di crescita epidermale)	25000	0.18	0.12	0.67
Fibronectina (integrina)	Fibronectina	50000	0.0007	0.6	860
Insulina	Insulina	10000	0.0096	0.2	21
TNF	TNF (citochina)	6600	0.93	0.14	0.15
Interleuchina 2	Interleuchina 2 (citochina)	200	1.89	0.014	0.0074

Considerare una coltura di condrociti seminati su scaffold porosi in microwells da 1.5 ml, con $1 \cdot 10^6$ cellule/scaffold. I condrociti esprimono circa 10^5 recettori per TGF- β , un fattore di crescita. A che concentrazione di TGF- β si ha il fenomeno di ligand depletion? K_D per il legame TGF- β - recettore per TGF- β e' 10^{-10} Molare.

La molecola di dexametasone (DEX) aumenta la produzione di collagene in osteoblasti, grazie all'interazione di DEX con un recettore. Per controllare la produzione di collagene in vivo e in vitro, si può utilizzare un farmaco che inibisce l'azione del DEX in maniera competitiva. La massima velocità di produzione di collagene è 100 molecole/cellula/s. In un tipico esperimento si aggiunge una concentrazione di $1 \cdot 10^{-8}$ M di DEX che dà luogo a una produzione del 75%.

a) Calcolare il K_D (costante di equilibrio) del DEX.,

b) si aggiunge poi il farmaco che inibisce la produzione di collagene. A $5 \cdot 10^{-7}$ M di farmaco, la produzione diminuisce al 65%. Calcolare il K_D per il farmaco inibitore..

c) Che concentrazione di DEX ci vuole per resituire la produzione di collagene in presenza di $5 \cdot 10^{-7}$ M del farmaco ?

d) quali assunzioni si fa per ottenere le soluzioni a, b, e c?.

Cell adhesion, cell cohesion

CAM :Cell Adhesion molecule, classified as one of generic CSR : cell surface receptors. Common names VCAM, PECAM.

Cells can adhere to each other (cohesion)

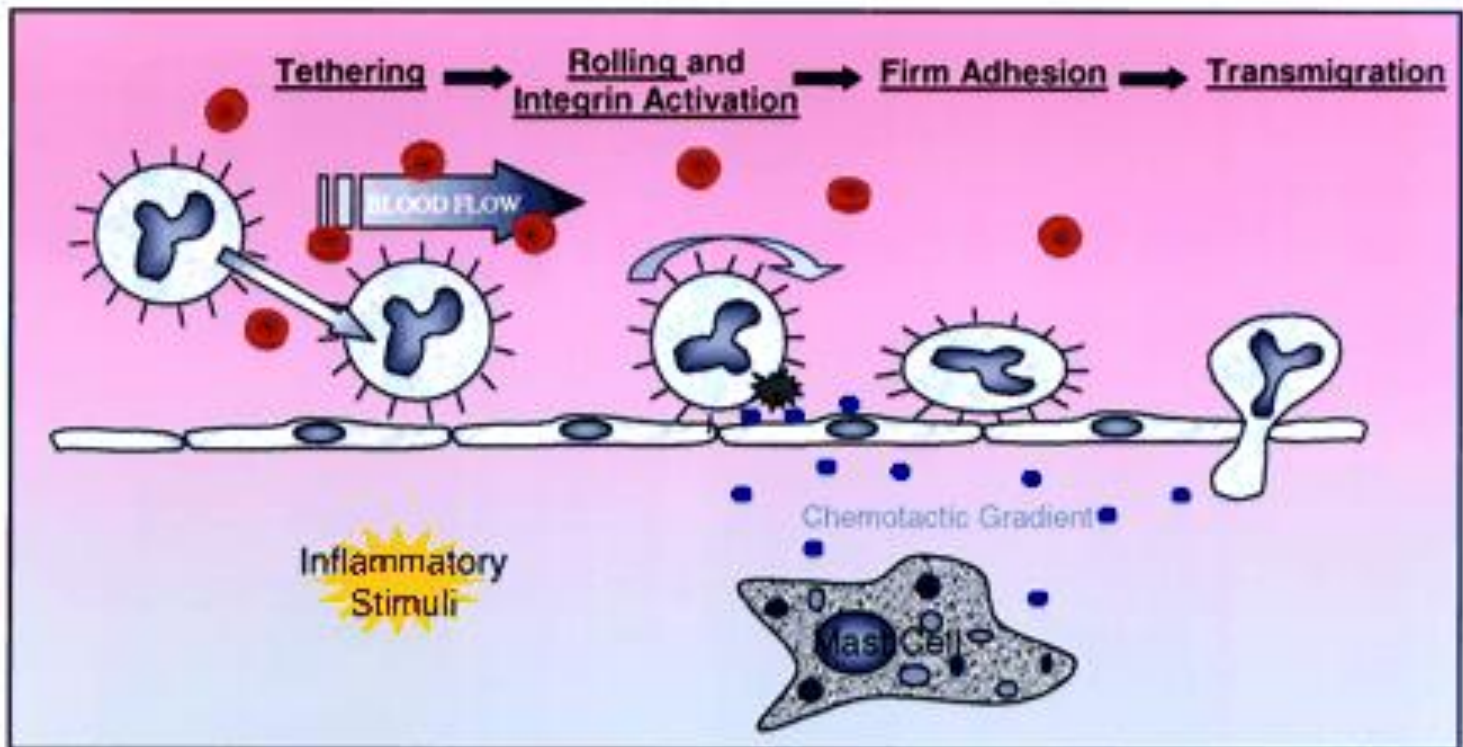
Or to the ECM (adhesion)

CAMs are responsible for structural integrity of adherent cells

Cell adhesion, cell cohesion: why?

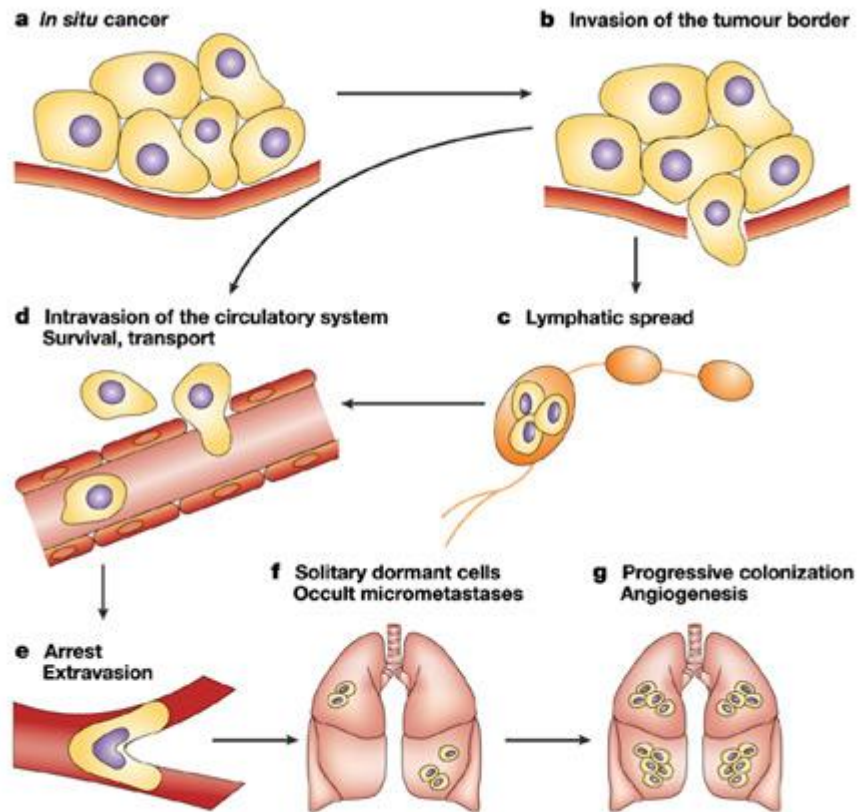
- Scaffold colonization
- Motility
- Metastasis
- Wound healing (scurvy)
- Morphogenesis
- Differentiation
- Inflammation and repair

Inflammatory response



Leucocyte rolling

Metastasis



There are 3 types of junctions between cells and cells or cells and ECM

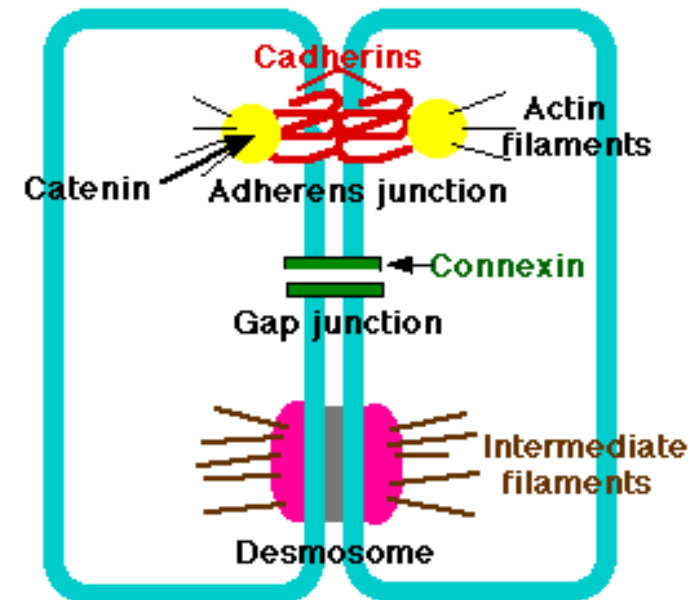
Tight junctions- especially in epithelial cells, they prevent diffusion of molecules

Communicating junctions – gap junctions, they regulate transport. For example in the liver and kidney

Anchoring junctions- they provide mechanical links- through integrins and cadherins



Linked to the cytoplasm
through cytoskeleton



The cytoskeleton: microfilaments, intermediate filaments and filaments.

Function: cell shape, motility, division, **mechanical strength (incompressible, resistant to tension)**.

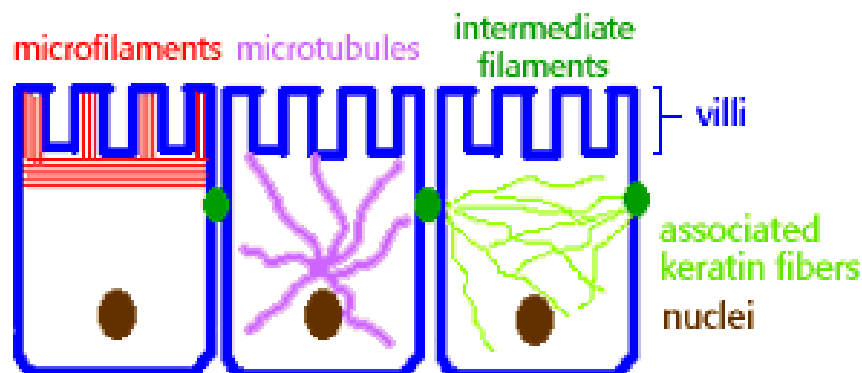
Micro filaments: Actin – contractile 3-6 nm

Intermediate filaments (fibrous proteins eg desmin, vimentin)- 10 nm. - tensile, rope like structures, much longer than actin. Form the structural framework in the cell.

Microtubules 25 nm. Cell shape and motility.

Tracks for vesicle movement

Cytoskeletal components of intestinal epithelial cells

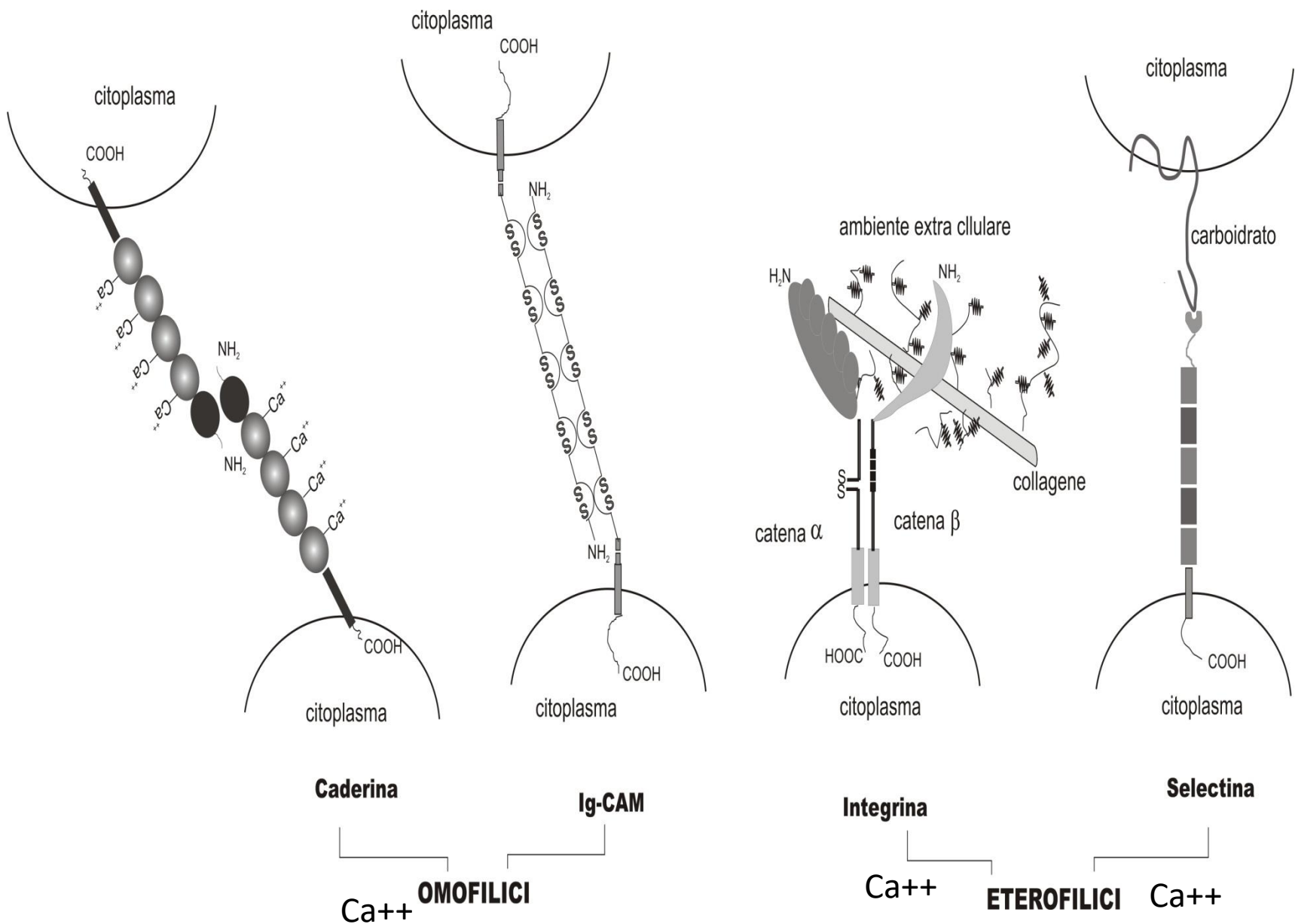


CAMs- junction proteins

Il meccanismo di riconoscimento attraverso i CAM è uno dei principali modi in cui la cellula interagisce con suo ambiente.

CAM	Caratteristiche
Integrine	-legano ai ligandi adesivi della matrice extra cellulare, sono detti legami eterofilici
Caderine	- legano a cellule vicine, generalmente omotipici (caderina-caderina) e sono calcio dipendenti. Le caderine sono fondamentali per la morfogenesi .
Ig CAM	- legano a altre cellule, generalmente formando legami omotipici, sono meno forti di legami caderine-caderine e sono le uniche CAM che non dipendono dalla presenza di calcio.
Selectine	- legano a mucine (la parte glicosata delle proteine), quindi formano legami eterofilici sono molto importanti per i processi infiammatorie.

Why are integrins not so important during early morphogenesis?



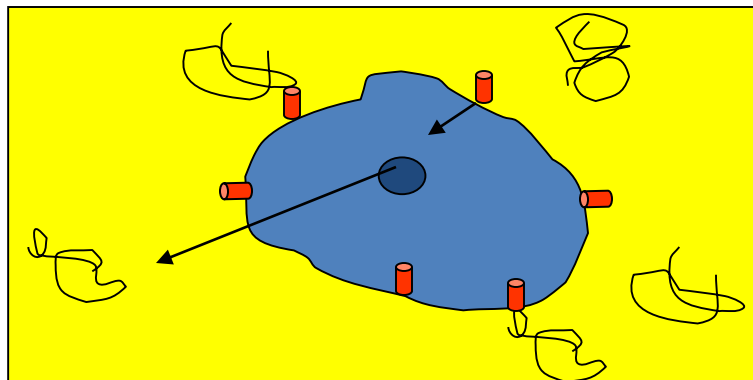
Le integrine

Adesione alla ECM

Trasduzione del segnale dal ECM alla cellula e dalla cellula al ECM

L'importanza dell'interazione tra cellule e la ECM. L' ECM non è solo una struttura di supporto ma gioca un ruolo attivo e importante in tante funzioni cellulari. Migrazione, proliferazione, differenziazione, apoptosis. Inoltre modula l'espressione delle citochine e i fattori di crescita e attiva la trasduzione e segnalazione intracellulare. Il rapporto cellule ECM funziona per reciprocità dinamica.

La ECM è l'ambiente che regola la dinamica dell'espressione genetica e differenziazione.



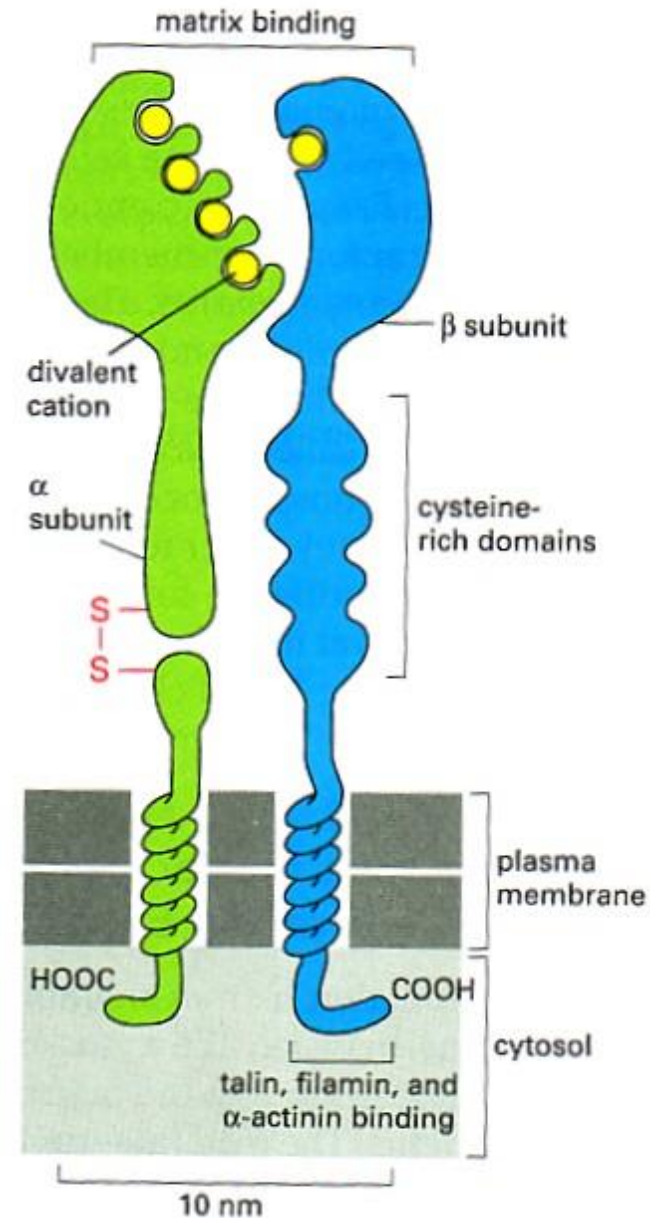
Le molecole del ECM interagiscono con i recettori (CSR-cell surface receptors, in particolare i CAM) che trasmettono segnali attraverso la membrana a molecole dentro il citoplasma. Questi segnali iniziano una cascata di eventi attraverso il CSK al nucleo (cytoskeleton) che risultano nell'espressione di geni. Questi vanno trascritti in proteine che hanno un effetto sull' ECM.

Le integrine

Sono delle proteine recettori che stanno sulla membrana cellulare, e modulano le interazioni tra cellula e la MEC. Tra queste interazioni, differenziazione, apoptosi, adesione, guarigione (“wound healing”). Insieme con le caderine, formano la famiglia di proteine recettori che sono coinvolte nelle interazioni cell-cell e cell-ECM.

Sono proteine transmembrane con 2 subunità glicoproteiche, α e β non legate cov. tra di loro. Si dice proteine eterodimeriche in cui le varie α e β sono omologhe fino al 40%. Per ora sono state identificate 9 subunità α e circa 16 β , e 24 integrine. Di queste 8 riconoscono Fn e 5 laminina. La catena α è più specifica nel riconoscimento.

Che differenza c'è tra la struttura delle integrine e quella dei anticorpi?

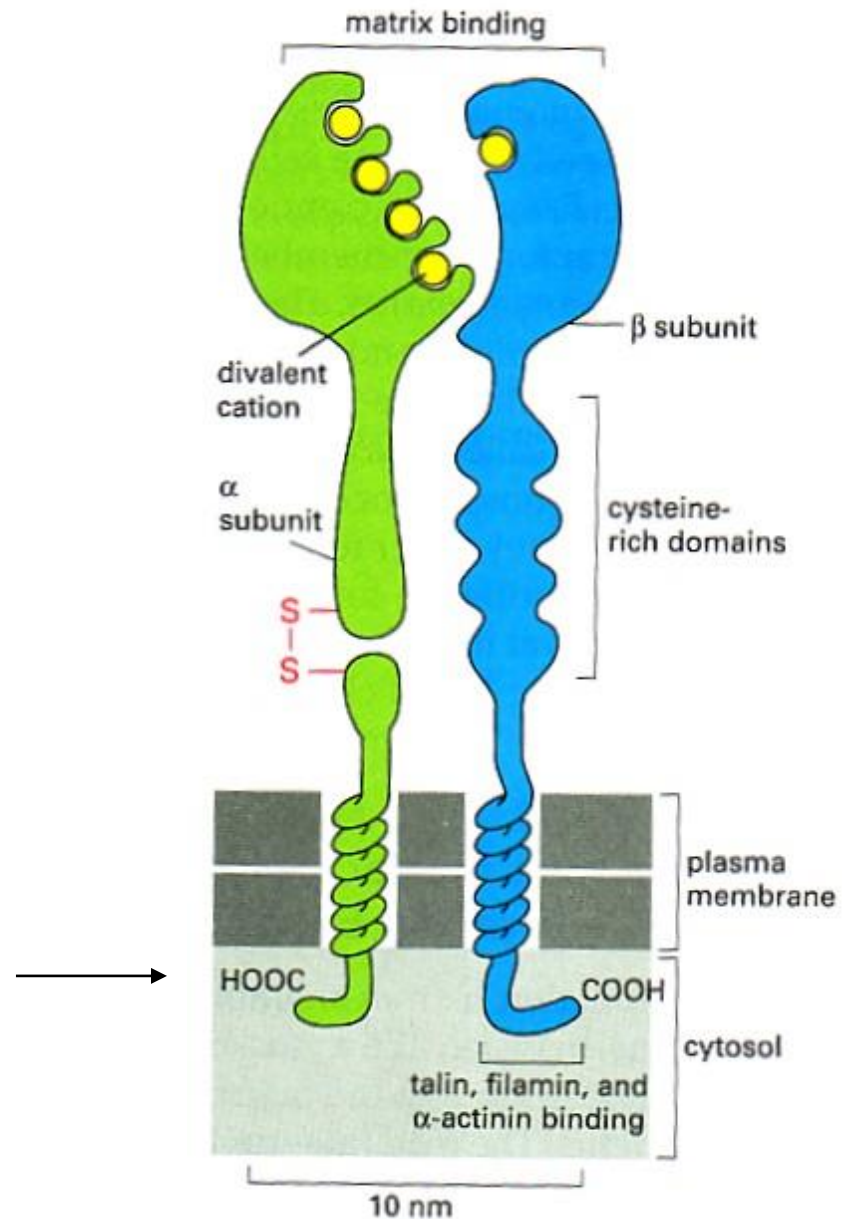


Alcune sequenze peptidiche sono riconosciute dalle integrine

- RGD (arginina-glicina-acido aspartico)
- YGISR (Tyr—Ile—Gly—Ser—Arg)
- E possono essere usate per decorare superfici di biomateriali per aumentare l'adesione cellulare

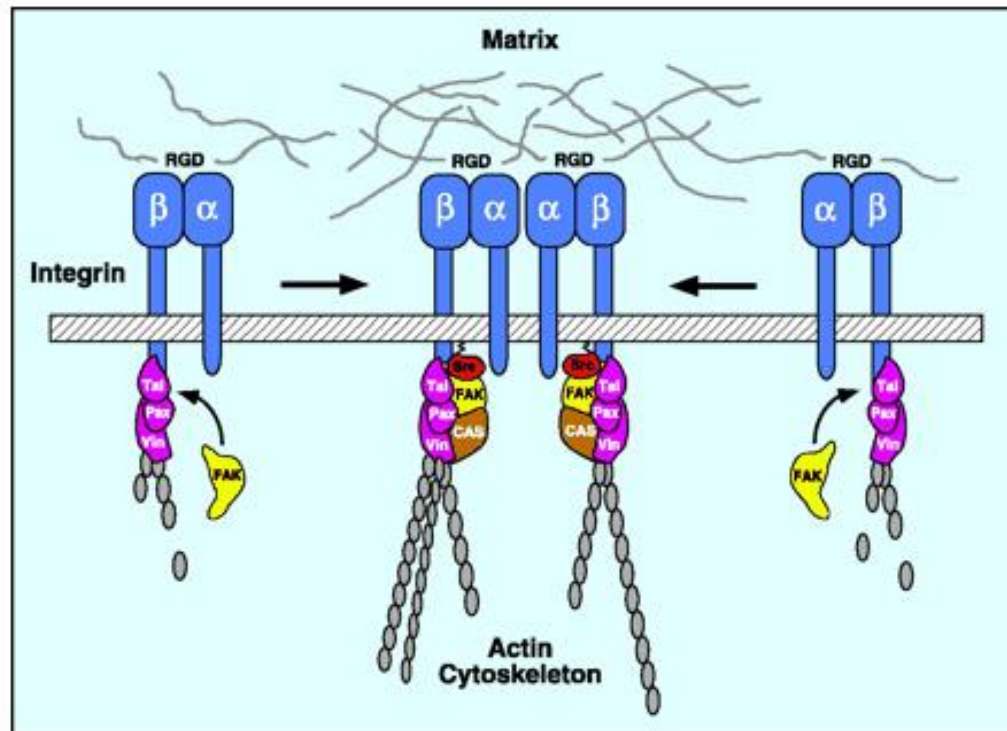
Integrin binding is calcium dependent. ECM proteins have specific amino acid sequences which bind to integrins. The most well known sequence is the RGD tripeptide (arginine, glycine, aspartic acid). Found in fibronectin, collagen, laminin. The alpha subunit is mainly responsible for recognition.

50 AA
residues



- Gran parte dell'integrina sta fuori nello spazio extra cellulare.
- La parte extra c. del dominio α ha 4 siti per legare a ioni $++$ e sono coinvolti nel legame calmodulina che è sempre presente nelle interazioni.
- Il legame con la ECM induce dei segnali intra cellulari. La parte interna interagisce con il CSK. *In generale, segnale dal ECM attraverso le integrine vengono trasdotte via il CSK e induce cambiamenti di forma che portano a movimento, proliferazione, differenziazione ecc.*
- Alcuni recettori sono specifici altri riconoscono più epitopi
- Possono anche diversificarsi (plasticità e ridondanza)
- $K_d = 10^{-6} - 10^{-9}$ moli/litro. Da confrontare con l'affinità anticorpo-antigene.
- Sono presenti in concentrazioni da 10 o più di 1000 per cellula. La loro azione dipende dalla concentrazione locale di ligandi, e possono solo agire quando presente in densità locali grandi (zone di adesioni focali o emidesmosomi). Quando sono diffusi in maniera omogenea sulla CM, non c'è adesione. Dopo alcuni stimoli, si raggruppano in contatti focali, e la somma delle loro affinità per unità di area aumenta. Le integrine possono spostarsi per espolarare l'ambiente. Se l'affinità fosse alta, non sarebbe facile interrompere il legame e non ci sarebbe motilità cellulare.
- Multiple weak adhesions-----few strong adhesions [440 nm e 160 nm]

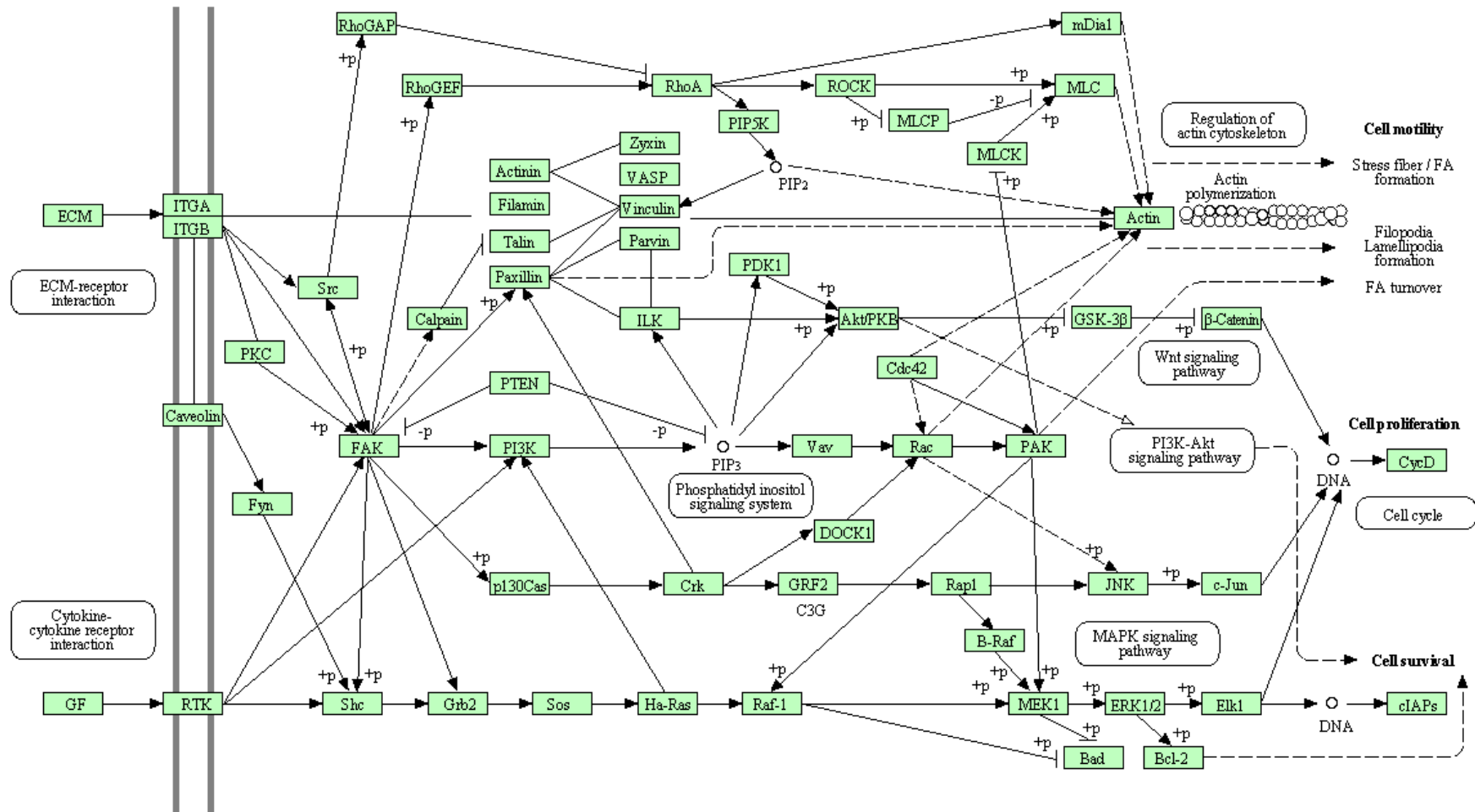
- Le Integrine si raggruppano e inizia una cascata di segnali
- *Focal adhesion kinase (FAK)*, un enzima tyrosina kinase è coinvolta
- FAK arriva agli contatti focali e viene fosforilato, iniziando una cascata di reazioni (quasi sempre di fosforilazione) che finiscono in una concentrazione di proteine nella zona focale.
- Il segnale viene trasmesso all'interno della cellula attivando l'organizzazione dello citoscheletro

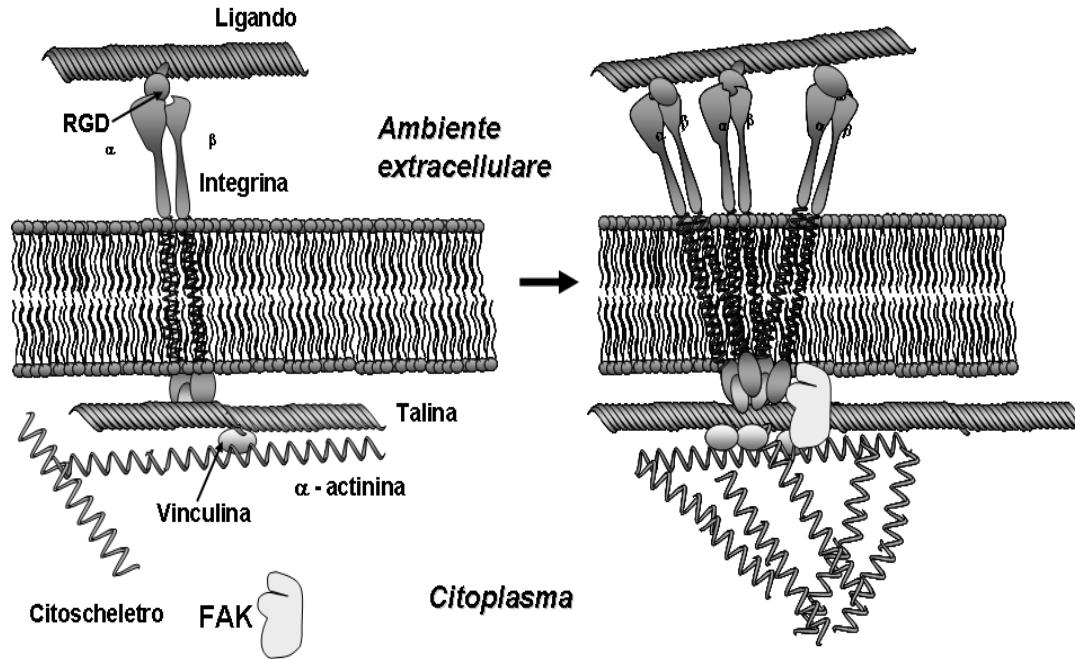


What is protein phosphorylation?

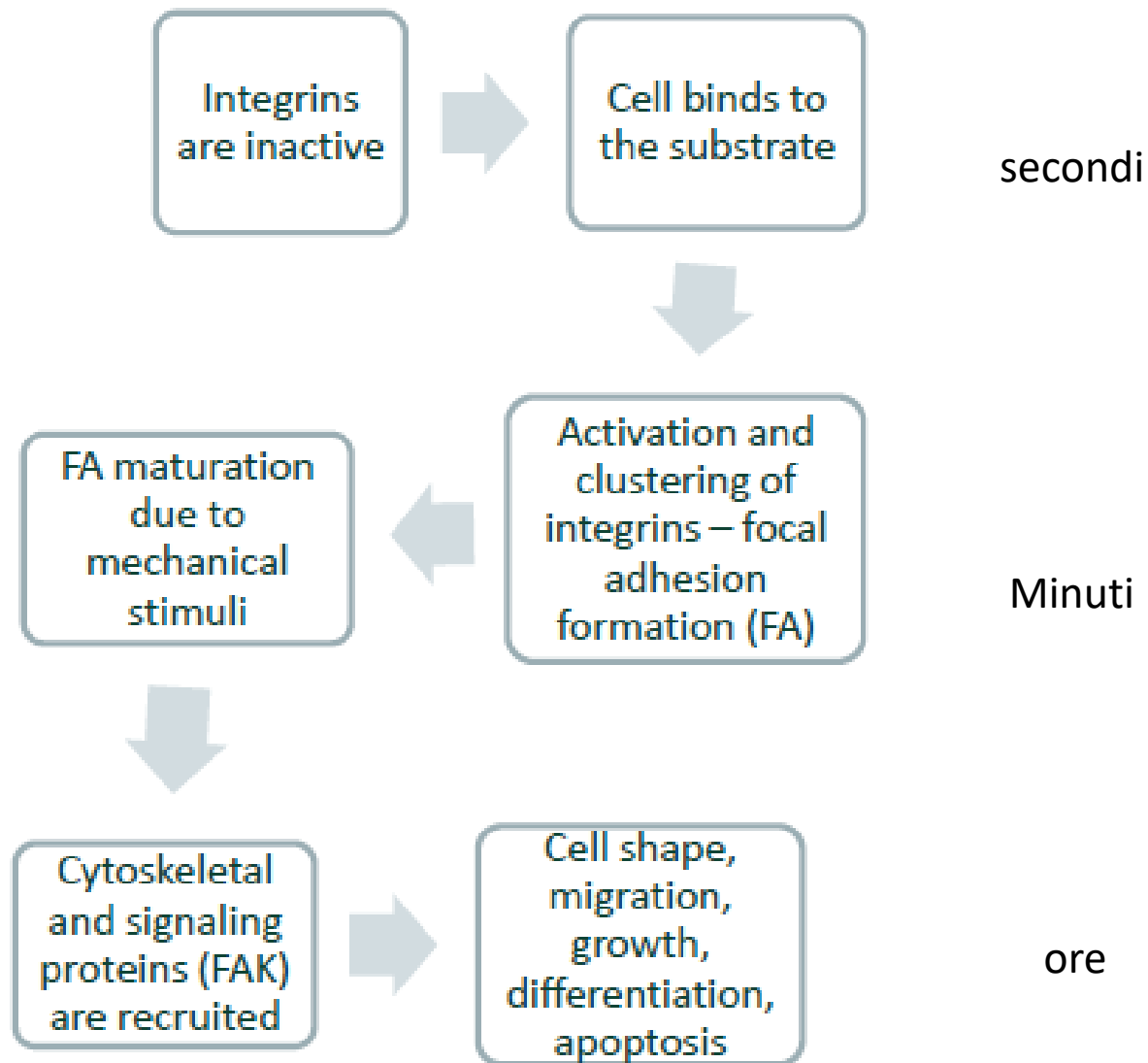
- Addition of a PO_4^{---} group to a protein. Makes the protein hydrophilic and polar, changes conformation. Thus activates other proteins (enzymes)
- Phosphorylation forms the basis of many enzymatic biochemical reactions.
- Amino Acids which phosphorylate are serine, tryosine, theronine
- It is a different to oxidative phosphorylation

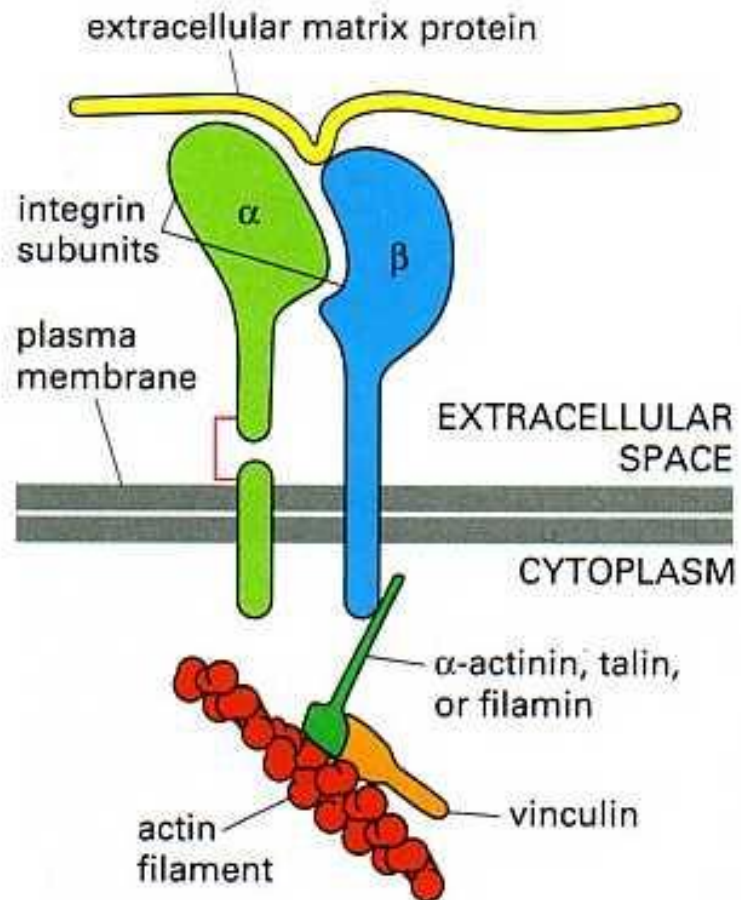
FOCAL ADHESION

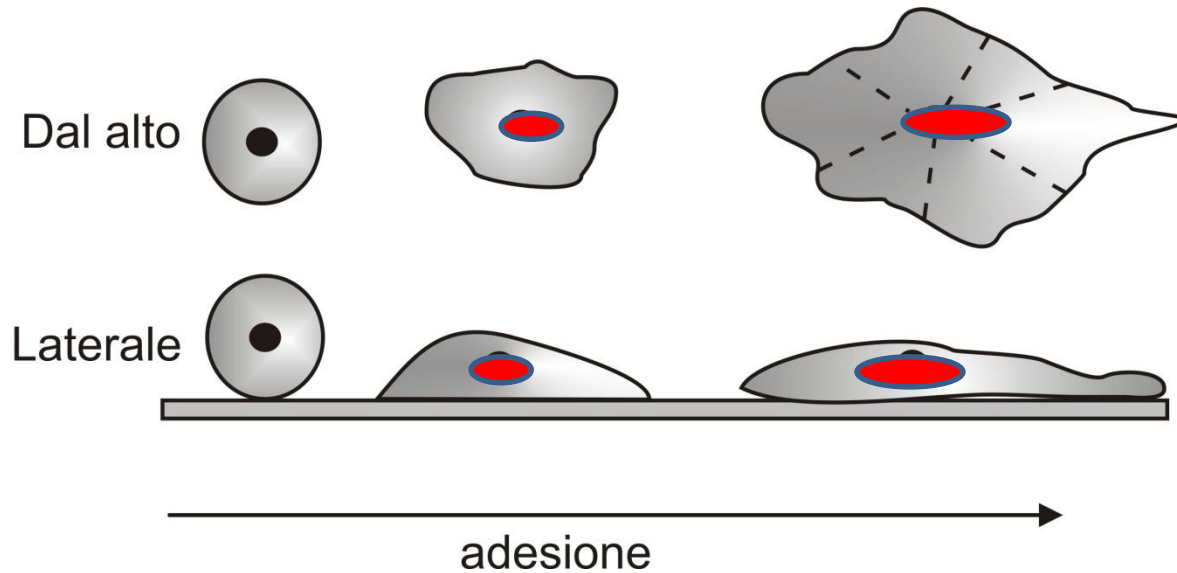




L'ancoraggio è accompagnato da un cambiamento di forma e proprietà meccaniche.







Cellula non adesa, poco adesa e molto adesa, su un substrato.

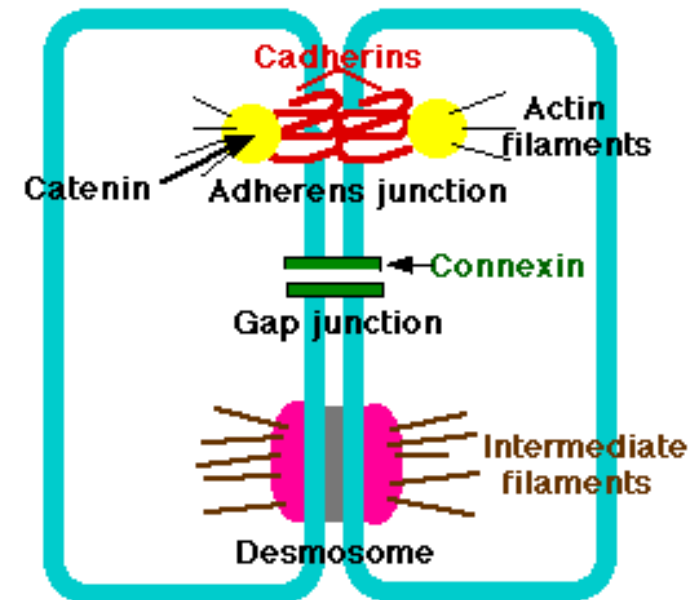
Ogni cellula aderisce in modo diverso a secondo del numero di integrine per cui ha anche forma diverso.

Forma e' altamente correlato alla funzione.

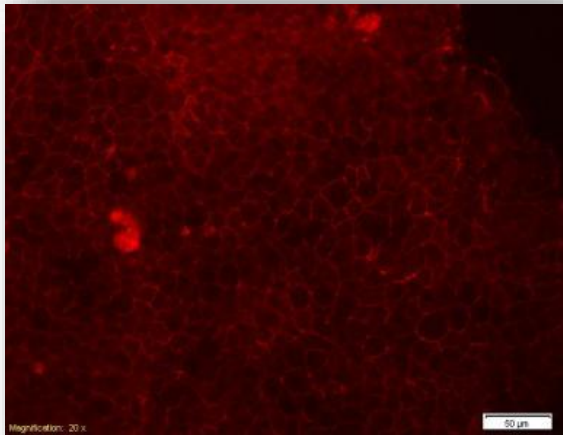
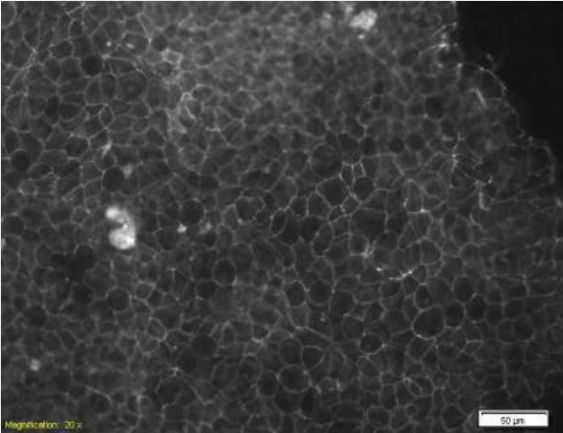
Le Caderine

Sono molecole presenti nei tessuti dei vertebrati e la loro azione dipende dalla presenza di calcio. Inizialmente sono state nominate in base al tessuto di appartenenza: caderina-E (epitelio), caderina-N (nervi) e caderina-P (placenta). Ogni tipo di cellula esprime un determinato set di caderine, che può cambiare se le funzioni della cellula cambiano. La porzione extracellulare è molto estesa e composta da cinque domini, di circa 100 amminoacidi ciascuno. Quattro di questi domini sono omologhi e contengono siti di legame per il calcio, ione indispensabile per la loro funzione. Solitamente le caderine sono impegnate in legami omofilici, di conseguenza, le caderine presenti sulla superficie di una cellula si legano alle caderine presenti sulle superfici cellulari. Diverse malattie sono associate con la disfunzione delle caderine.

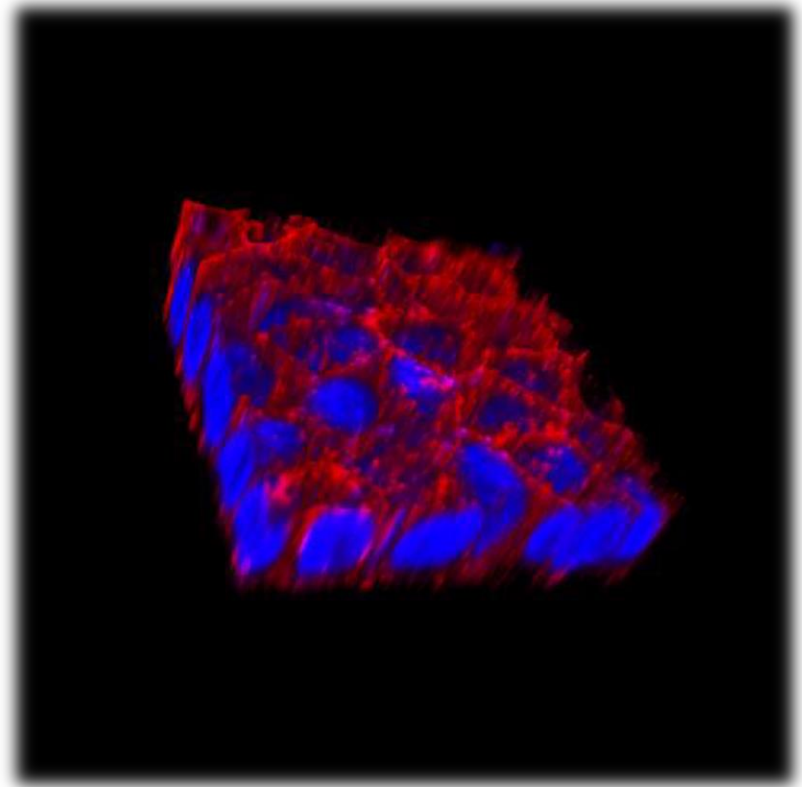
adiacenti. La loro affinità è piuttosto bassa, e il principio di funzionamento è simile a quella delle integrine.



Intestinal epithelia



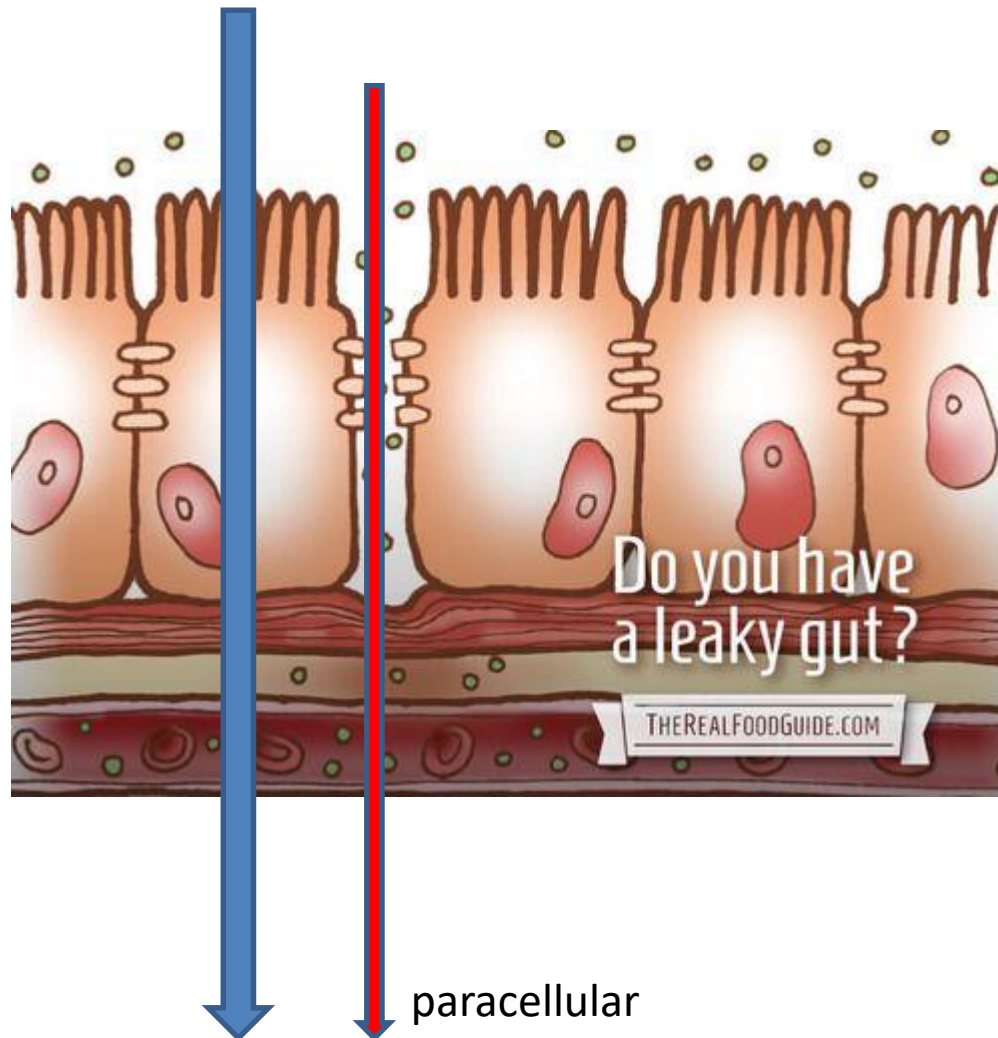
20X



Tight junctions :red. Nuclei:blue

transcellular

TEER-
trans-
epithelial-
electrical
resistance



Tight junctions

Integrins
Basal lamina

paracellular

Le Ig-CAM

Questi recettori assomigliano agli anticorpi e sono importanti nella regolazione fine della coesione, soprattutto nell'embrione. (Ca⁺ indipendente)

Le Selectine

Le selectine possiedono un dominio capace di legarsi ai carboidrati e giocano un ruolo nella risposta infiammatoria perchè in genere si legano agli zuccheri presenti sulla superficie dei neutrofili. .

Le caderine sono importanti per lo sviluppo aggregazione e disaggregazione cellulare (up and down regulation).

Anticorpi contro cadherina rompono i legami e distruggono epitelio

Sono Ca dipendenti

E cad (epiteliale), N cad, ecc



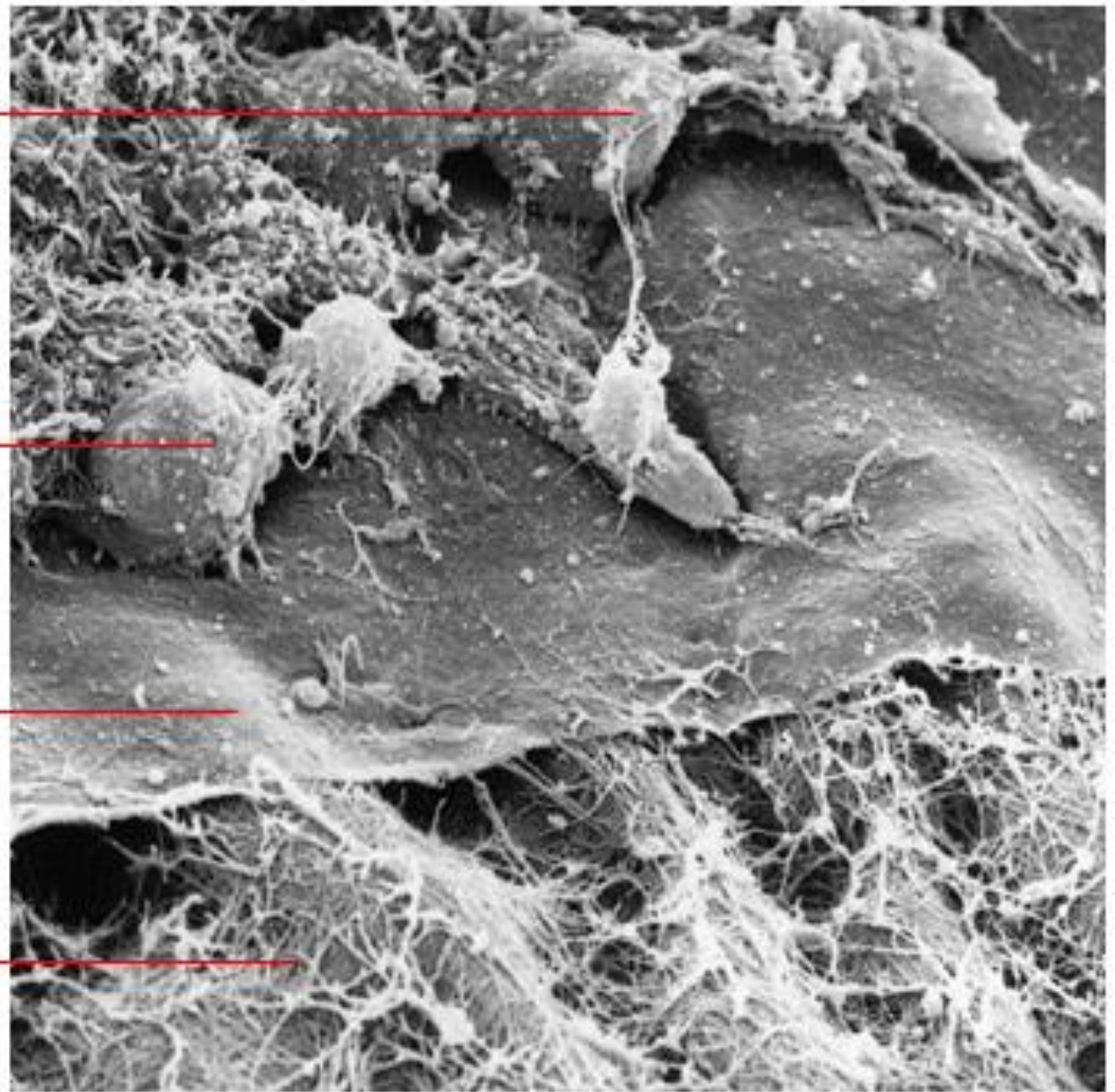
I IgCAM (LCAM e NCAM) invece non sono Ca^{++} dipendenti, meno forti delle caderine, e possono cambiare la forza del legame riducendo la lunghezza della catena extra citoplasmica che va a interagire con la IgCAM di un'altra cellula

Epithelial cells

Epithelial cells

Basal lamina

Collagen fibrils



10 μm

Scanning electron micrograph of a basal lamina in the cornea of a chick embryo

Stimare il numero di integrine che una cellula endoteliale deve possedere per superare le forze di taglio imposte dal flusso sanguigno in un'arteria.

Diametro cellula= 20 μm , altezza trascurabile

Velocità media del sangue nell'arteria di diametro 1.5 cm= 40 cm/s

Viscosità del sangue=0.004 Pas

Usare i dati per il problema sotto.

Uno dei problemi associato all'utilizzo di scaffold sintetici per l'ingegnerizzazione dei vasi è la mancanza di un'adeguata adesione di cellule endoteliali sulla parete luminale, che causa la formazione di trombi e altre complicazioni. Uno degli approcci considerati è l'immobilizzazione di ligandi di adesione, tipicamente in forma di sequenze amminoacidi contenenti RGD. Dato che un'integrina si lega a un RGD, calcolare la densità superficiale ($\#/\mu\text{m}^2$) di RGD necessario per assicurare un'adeguata adesione e quindi la distanza tra un ligando e l'altro. Discutere alcuni dei problemi che si possa incontrare con l'utilizzo di questo approccio. I dati sono da confrontare con le densità superficiali di 600-700 ligandi/ μm^2 necessarie per formare adesioni focali riportate in Cavalcanti-Adam et al (Cell Spreading and Focal Adhesion Dynamics Are Regulated by Spacing of Integrin Ligands, Biophysical Journal, Volume 92, 2007 p. 2964–2974).