Ανίχνευση βιοδραστικότητας διατροφικών συμπληρωμάτων σε in vivo και in vitro μοντέλα ασθενειών του ανθρώπου και η επίδρασή τους στο μικροβίωμα

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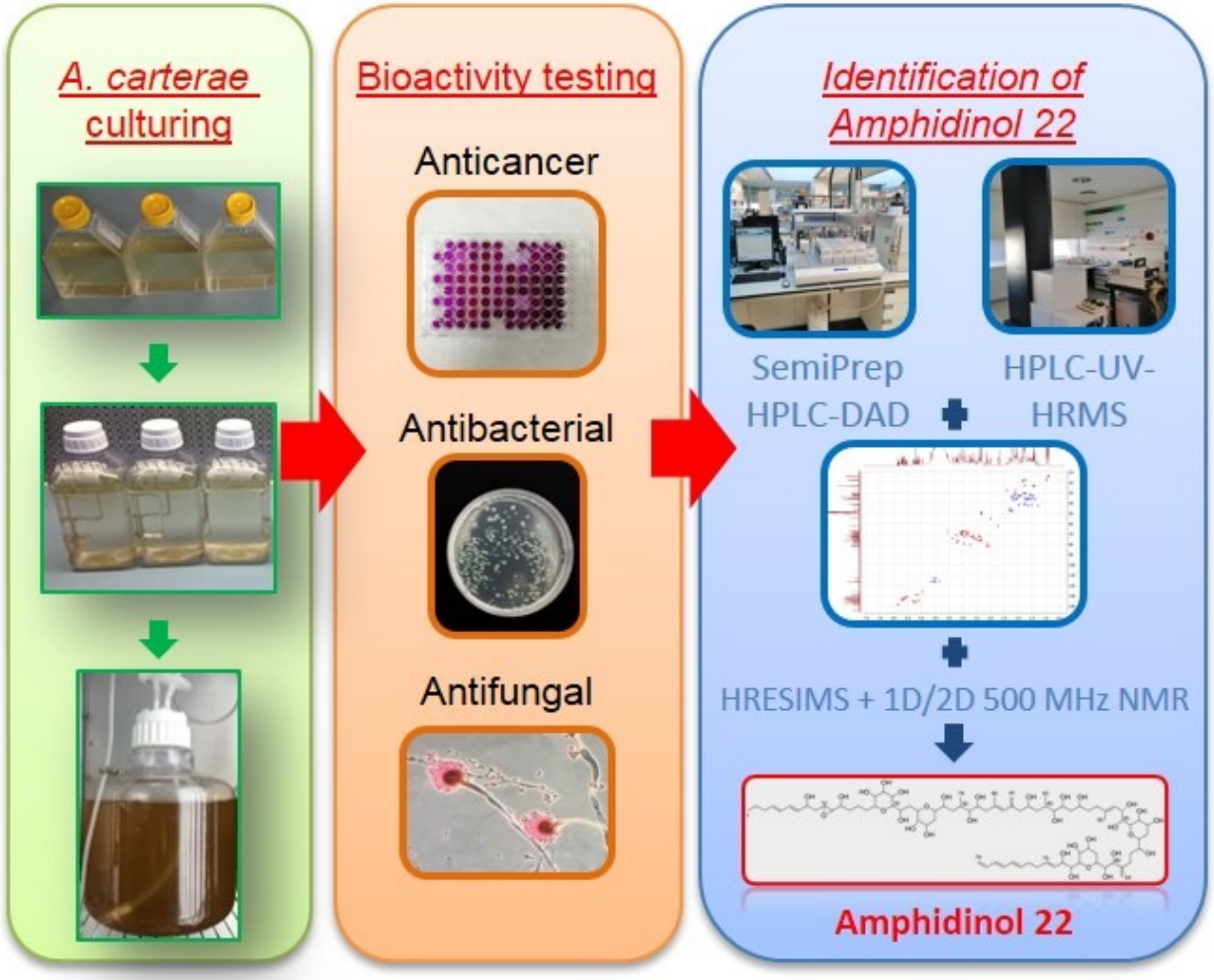
Ελεγχος βιοδραστικότητας

- Μοντέλα κυτταροκαλλιεργειών
- Μοντέλα πειραματοζώων
- Κλινικές μελέτες
  - Προσοχή στη μεθοδολογία!!

Bioactive compounds from different sources have shown anti-cancer activity by directly targeting cancer stem cells.


A. carterae culturing
Bioactivity testing
Anticancer
Antibacterial
Antifungal
Identification of Amphidinol 22
SemiPrep HPLC-DAD
HPLC-UV-HRMS
HRESIMS + 1D/2D 500 MHz NMR
Amphidinol 22

Ελεγχος βιοδραστικότητας

- Μοντέλα κυτταροκαλλιεργειών
  - ηπατοκυττάρων
  - εντερικού επιθηλίου
  - πνευμονικού επιθηλίου
  - μακροφάγων
  - Τ κυττάρων

Cell culture

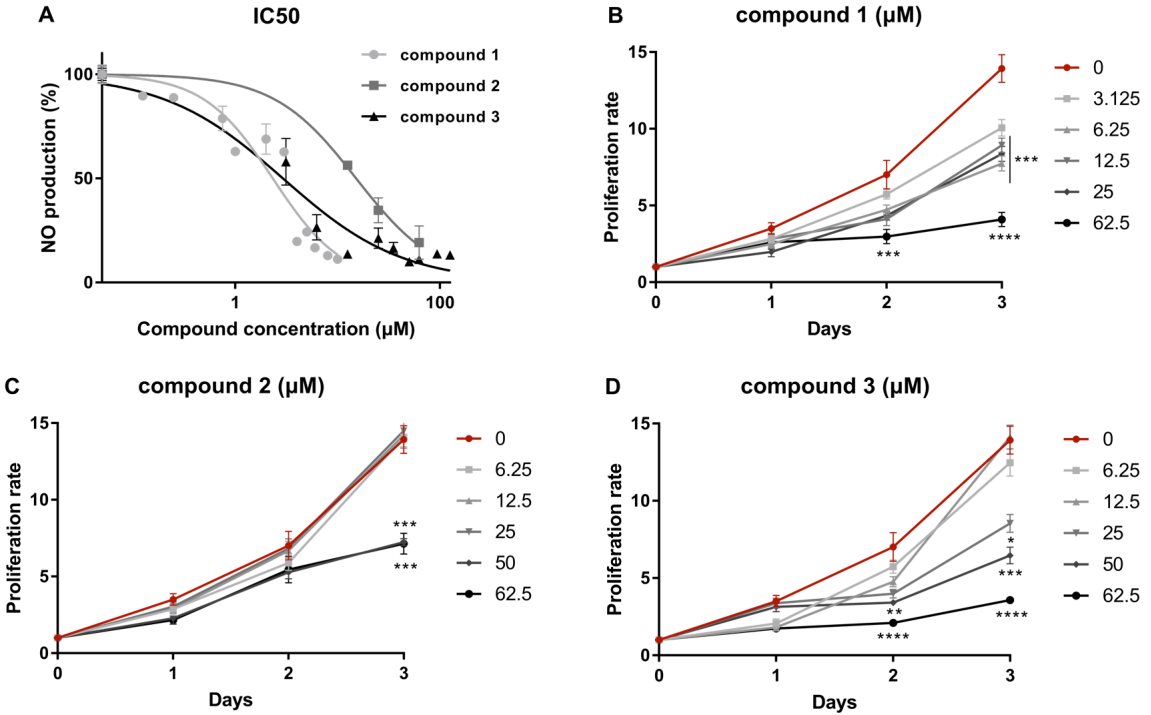
Treatment with extracts with potential biological activity

Evaluate bioactivity

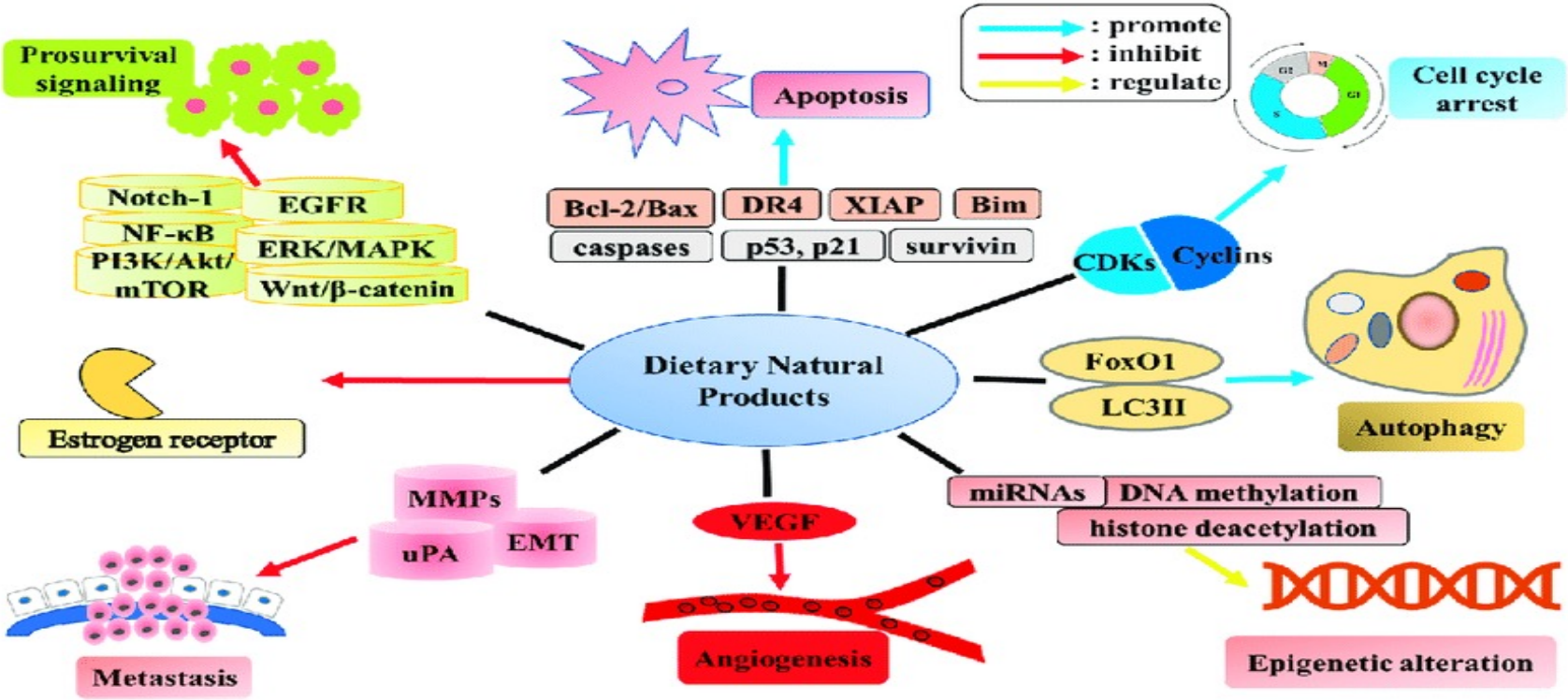
Measure proliferation and metabolic capacity using MTT assay

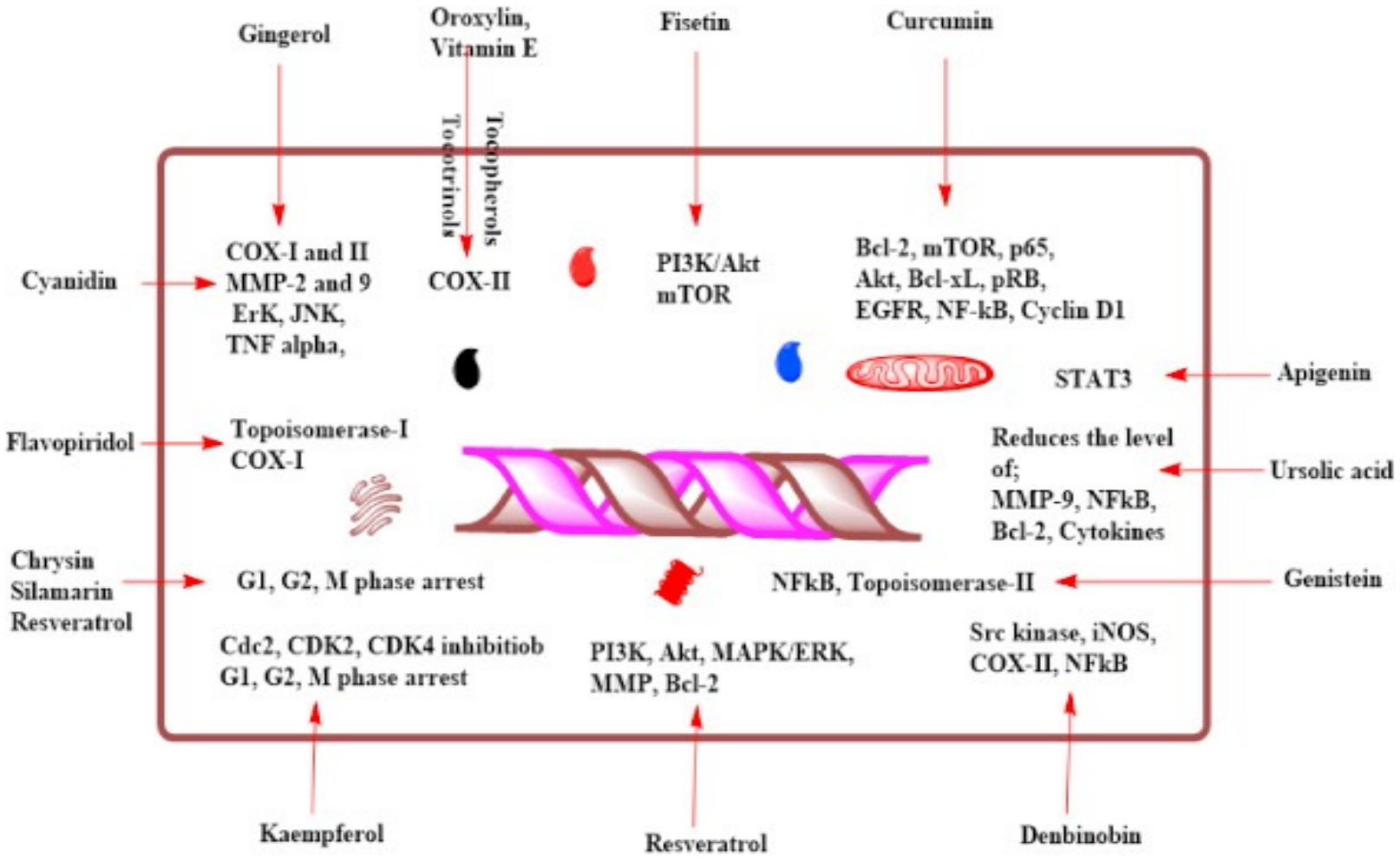
Αντικαρκινικη δράση εκχυλισμάτων σε μοντέλα κυτταροκαλλιέργειας

Εκτίμηση κυτταροστατικότητας εκχυλισμάτων



Κατανόηση μηχανισμού δράσης και μοριακής σηματοδότησης

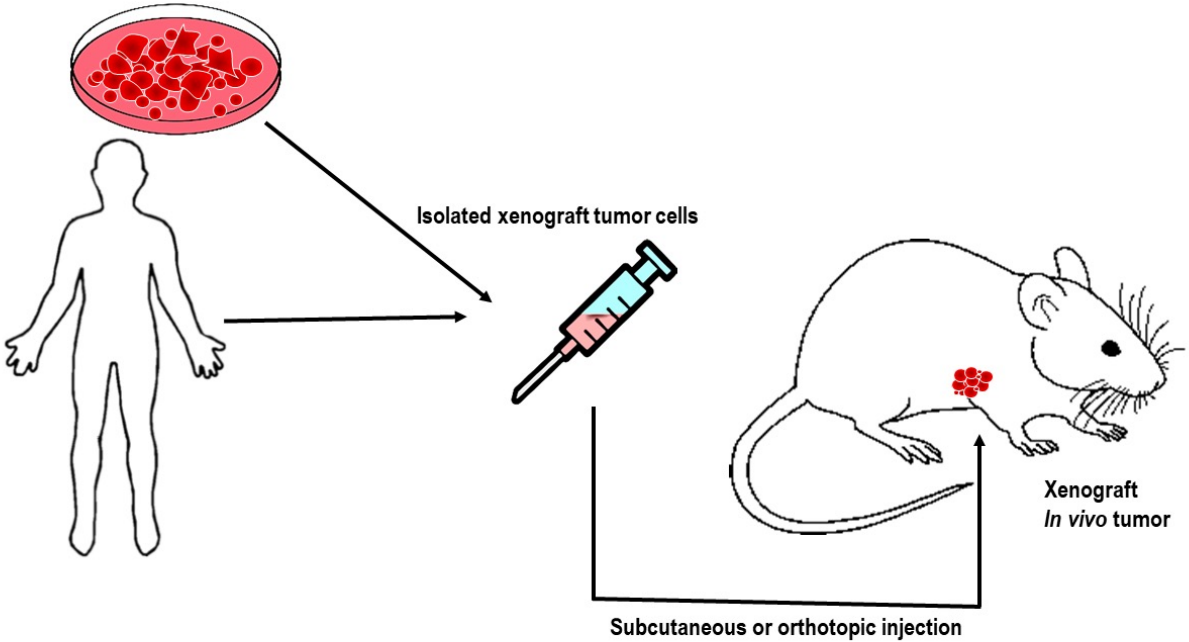



Gingerol
Oroxylin,
Vitamin E
Fisetin
Curcumin
Tocotrinols
Tocopherols
Cyanidin
COX-I and II
MMP-2 and 9
ErK, JNK,
TNF alpha,
COX-II
PI3K/Akt
mTOR
Bcl-2, mTOR, p65,
Akt, Bcl-xL, pRB,
EGFR, NF-kB, Cyclin D1
STAT3
Apigenin
Flavopiridol
Topoisomerase-I
COX-I
Reduces the level
of;
MMP-9, NFkB,
Bcl-2, Cytokines
Ursolic acid
Chrysin
Silamarin
Resveratrol
G1, G2, M phase arrest
NFkB, Topoisomerase-II
Genistein
Cdc2, CDK2, CDK4 inhibitiob
G1, G2, M phase arrest
PI3K, Akt, MAPK/ERK,
MMP, Bcl-2
Src kinase, iNOS,
COX-II, NFkB
Kaempferol
Resveratrol
Denbinobin

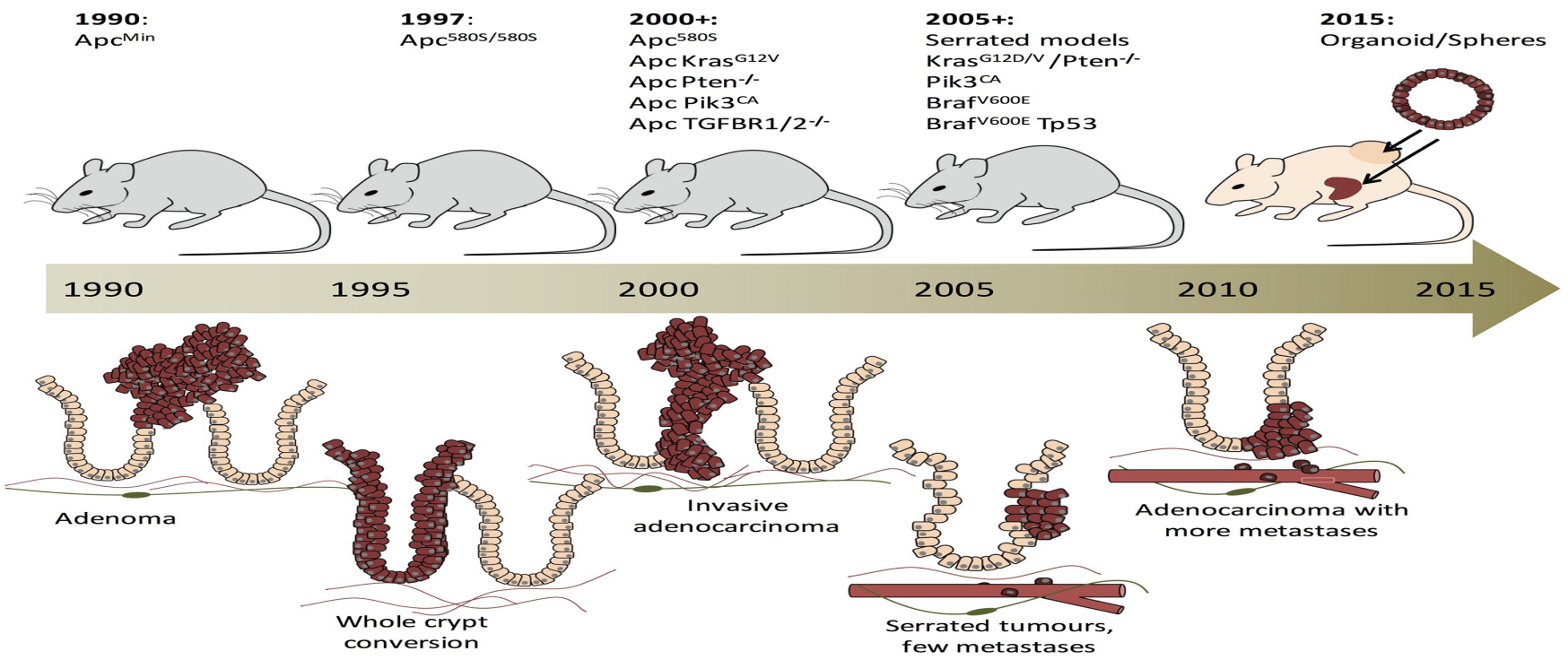
Βιοδραστικές ενώσεις δρούν σε κυτταρικά μονοπάτια

- Βιοδραστικές ενώσεις παρεμποδίζουν τις COX (1 και 2)
- Δρούν στον κυτταρικό μεταβολισμό (μέσω PI3k/Akt/mTOR)
- Επηρεάζουν τον κυτταρικό κύκλο (πχ cyclins/CDKs)
- Επηρεάζουν τους μηχανισμούς απόπτωσης

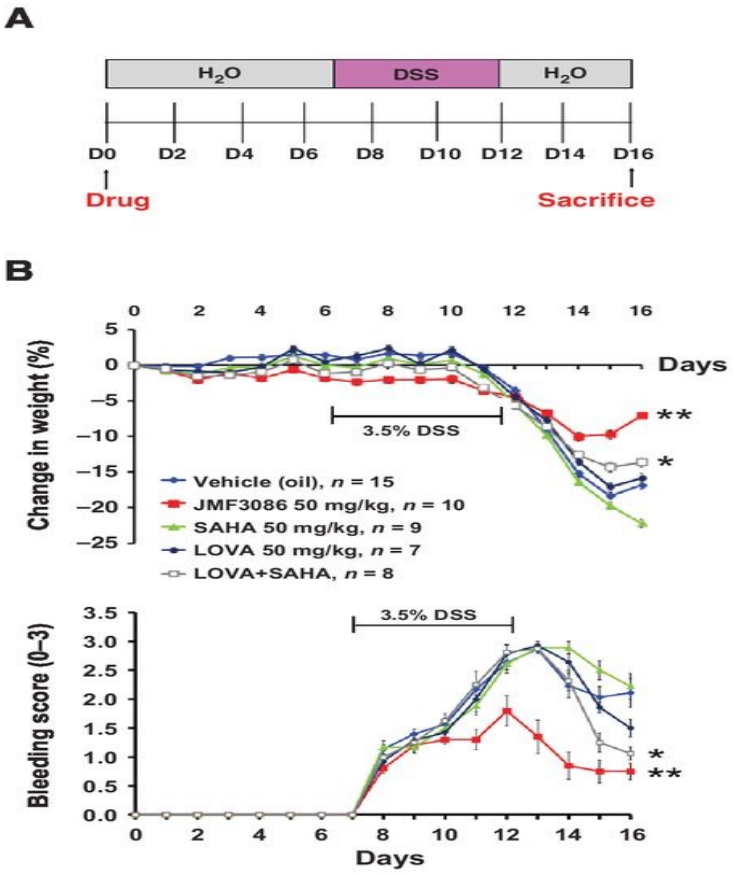
In vivo μοντέλα εκτίμησης βιοδραστικότητας κατά του καρκίνου: xenografts



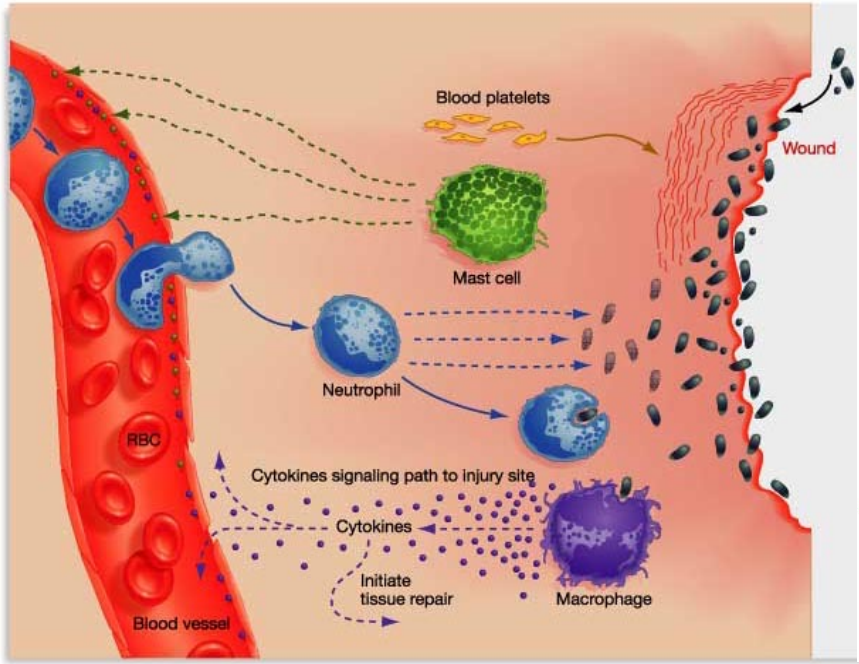
In vivo μοντέλα εκτίμησης βιοδραστικότητας: γενετικά μοντέλα καρκίνου

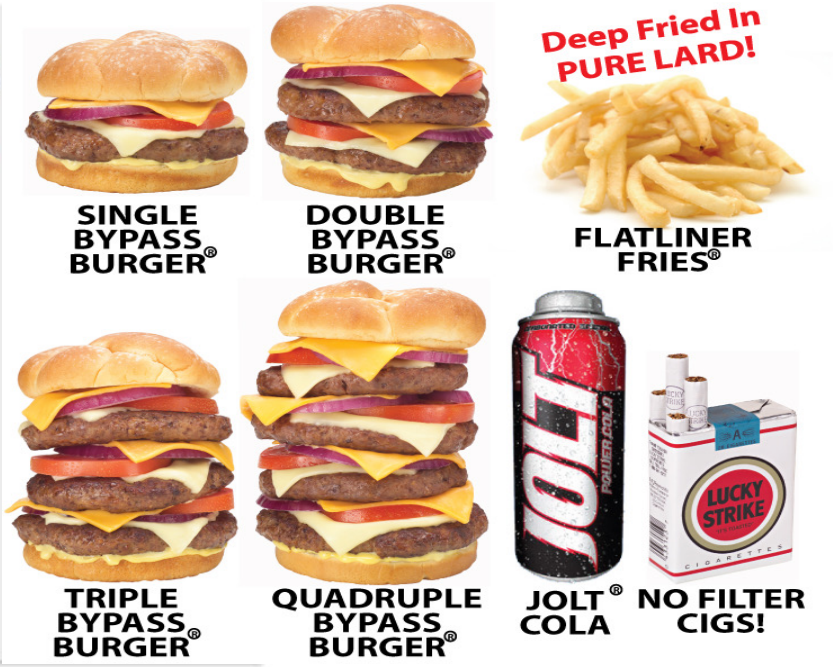


Μοντέλα χημικής καρκινογένεσης

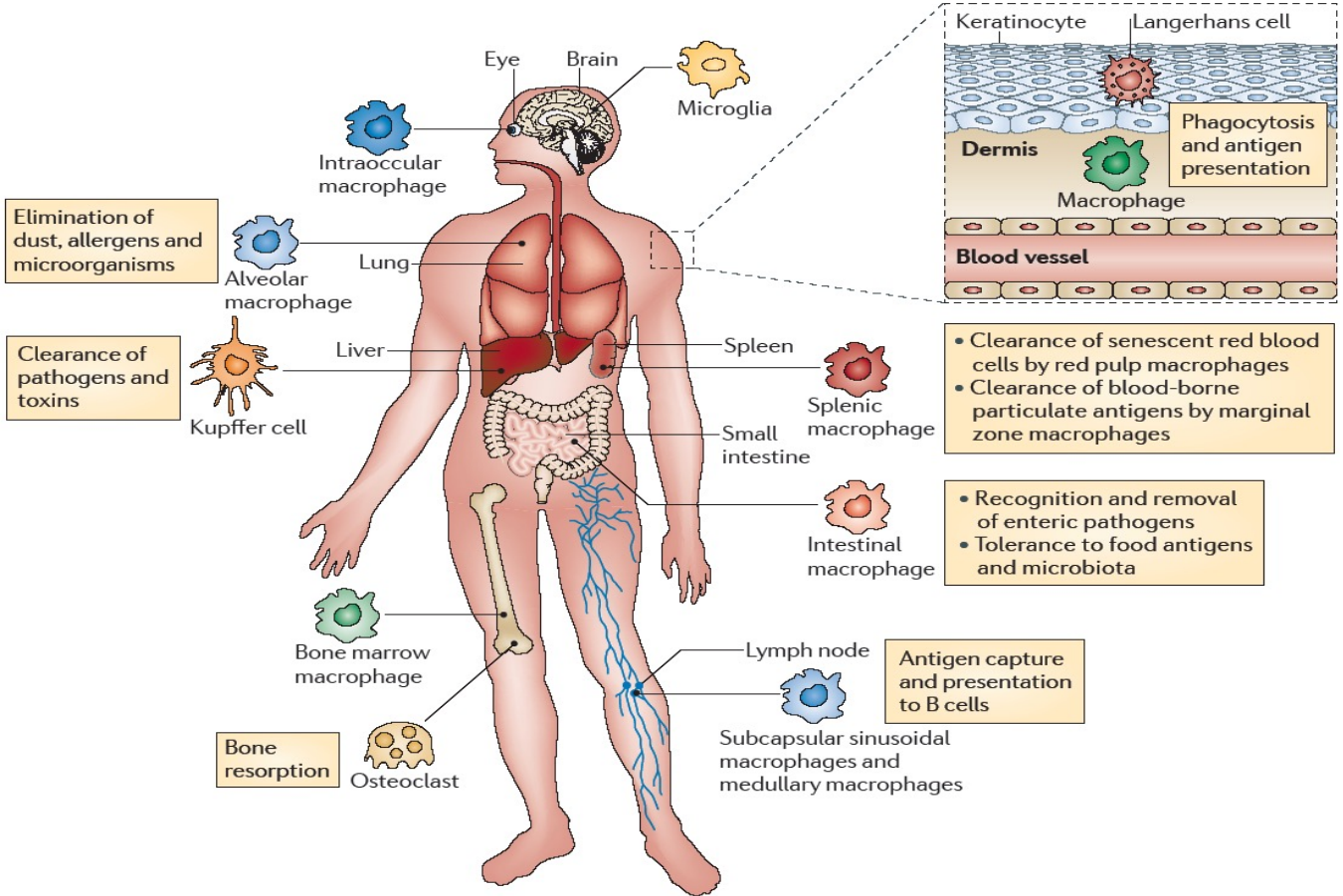


Εκτίμηση αντιφλεγμονώδους δράσης

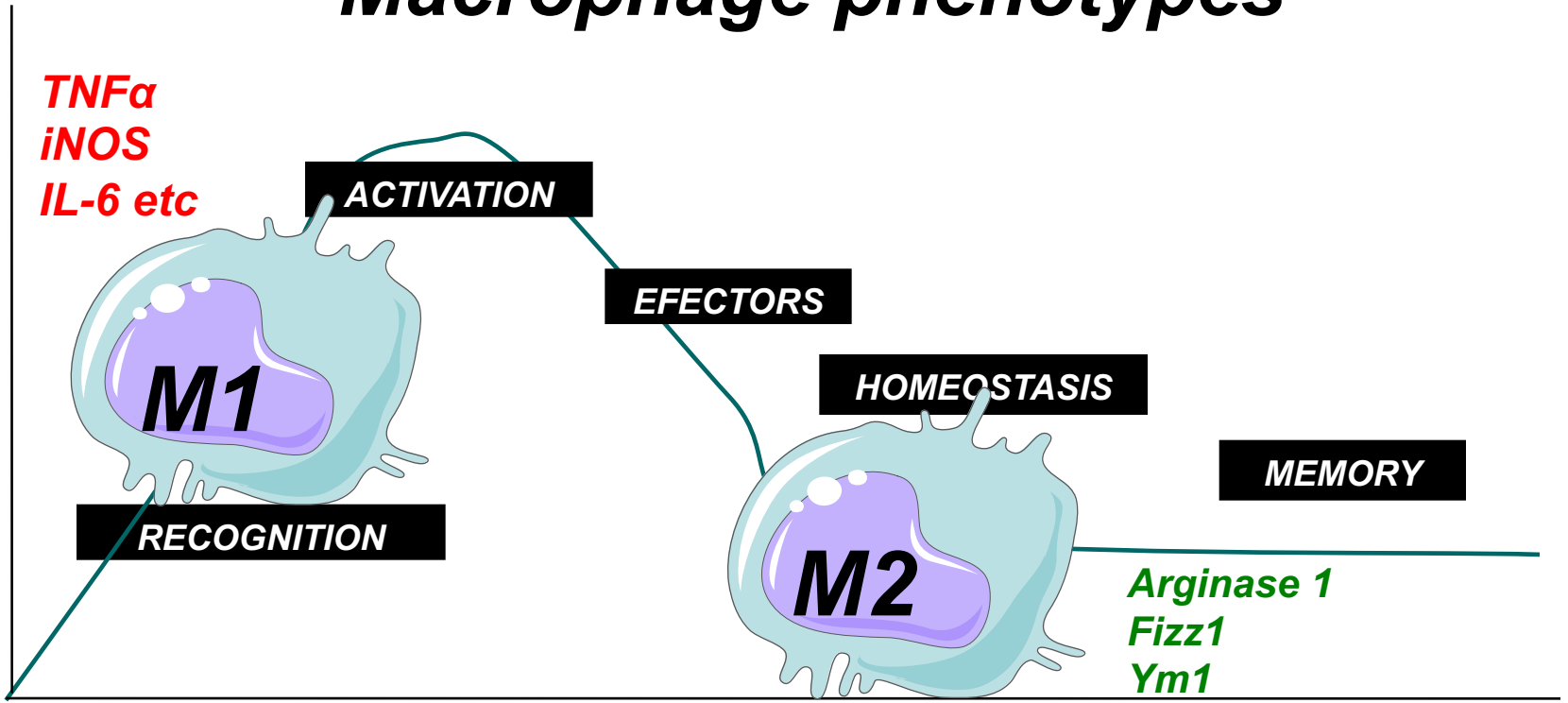




Macrophages - gatekeepers

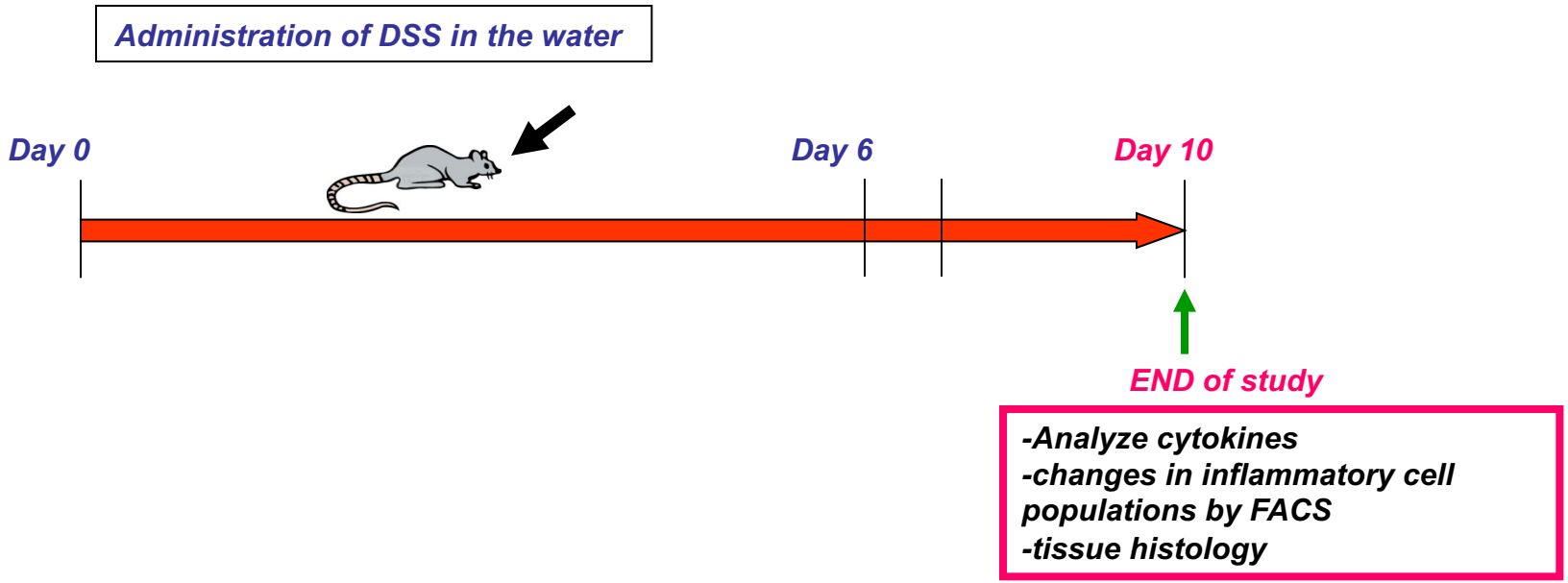


Macrophage phenotypes

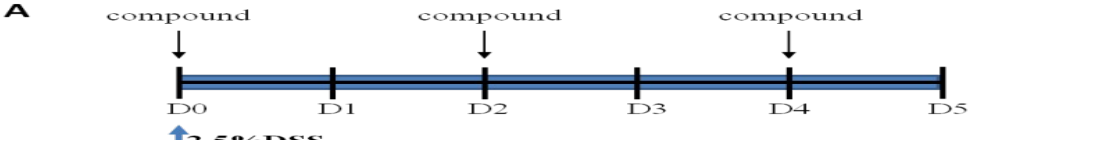


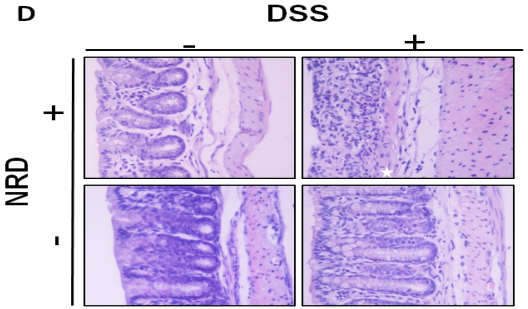
Μελέτη αντιφλεγμονώδους δράσης σε ελκώδη κολίτιδα

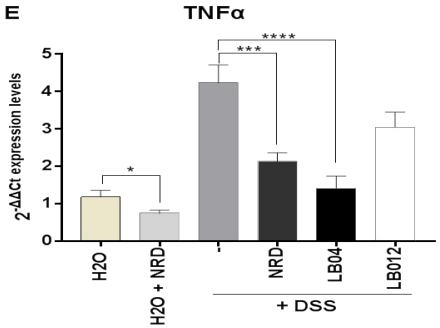
DSS-induced inflammatory bowel disease

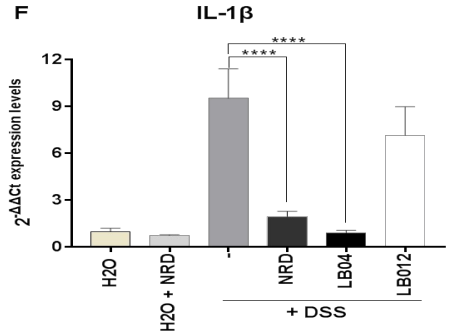


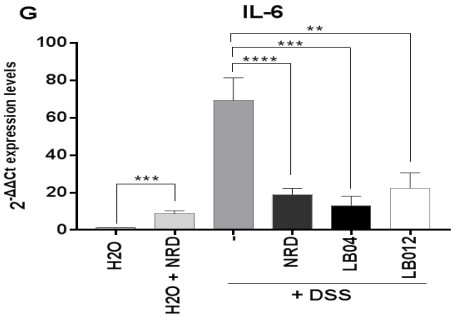
Μελέτη της επίδρασης εκχυλισμάτων φυκών στην εντερική φλεγμονή χρησιμοποιώντας το μοντέλο της ελκώδους κολίτιδας



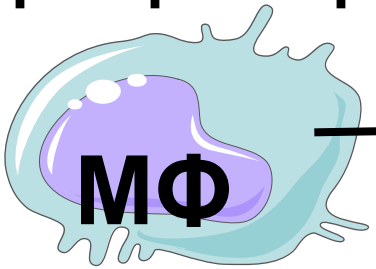






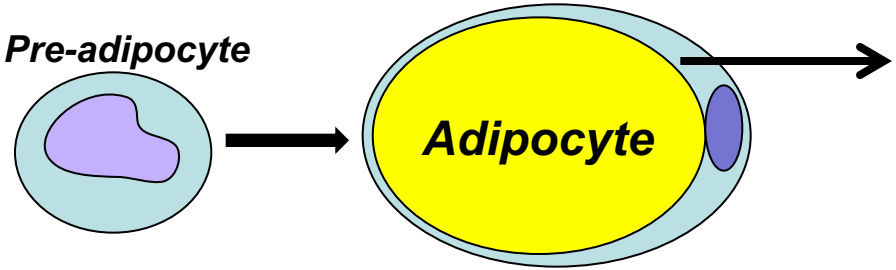


Φλεγμονή: κεντρικός διαμεσολαβητής είναι τα μακροφάγα



Nitric Oxide
Προ-φελγμονώδεις Κυτταροκίνες
Αντι-φλεγμονώδεις Κυτταροκίνες

Μεταβολική φλεγμονή: ο ρόλος των λιποκυττάρων

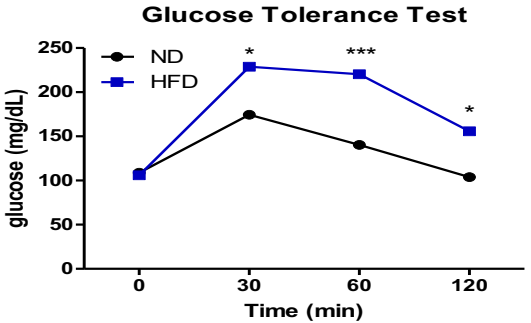


Προ-φελγμονώδεις Κυτταροκίνες
Λιποκίνες (leptin, adiponectin etc)

Ανάλυση βιοδραστικότητας εκχυλισμάτων σε μεγάλη κλίμακα για αντι-φλεγμωνώνδεις και αντι-λιπογόνες δράσεις
Επιλογή εκχυλισμάτων για εκτενή ανάλυση βιοδραστικότητας σε μικρή κλίμακα και in vivo αναλύσεις

Εκτίμηση της αντι-φλεγμονώδους και αντι-λιπογόνου δράσης σε μοντέλο διαβήτη τύπου 2 και μεταβολικής φλεγμονής μέσω δίαιτας με υψηλά λιπαρά σε ποντίκια

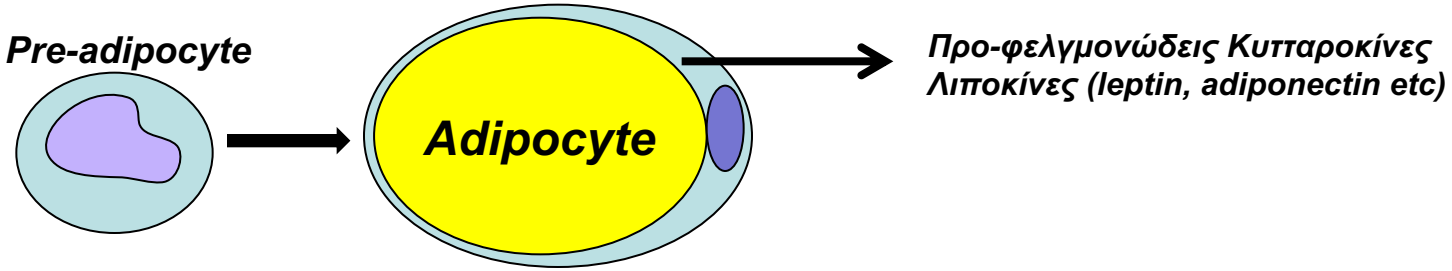
Diet	Mean Body Weight in g. +/- SEM
Long term HFD (N=10) *	34.11 ± 1.395
Long term ND (N=8)	24.98 ± 0.4825



Ανάλυση βιοδραστικότητας και αξιολόγηση οργανικών εκχυκλισμάτων μικρής κλίμακας

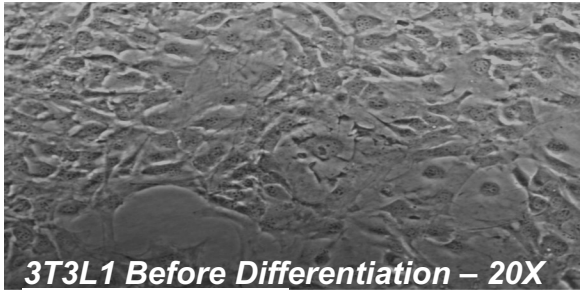
Εκτενής αξιολόγηση βιοδραστικότητας απομονωθέντων φυσικών προιόντων (ή επιλεγμένων κλασμάτων) απο εκχυλίσματα

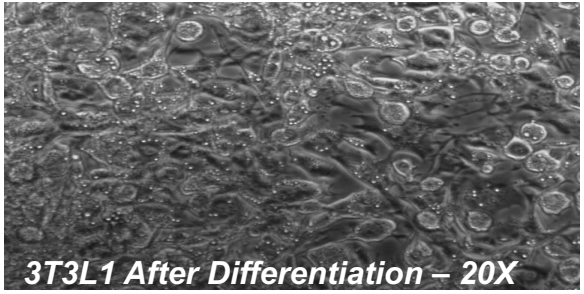
Μελέτη δραστικότητας εκχυλισμάτων στην διαφοροποίηση λιποκυττάρων και την παχυσαρκία σε μοντέλο παχυσαρκίας

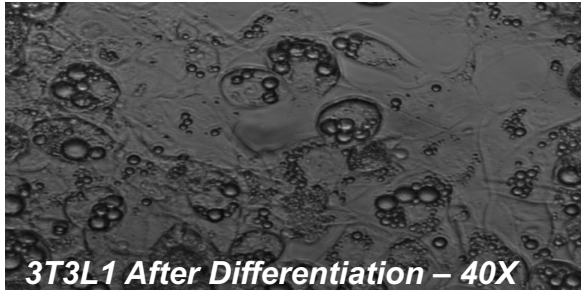


3T3L1 cells as adipocyte differentiation model

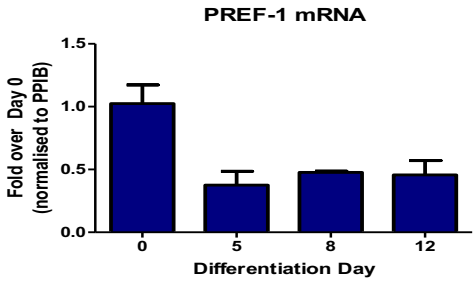
3T3L1 pre-adipocytes + Differentiation cocktail → adipocytes after 2 weeks

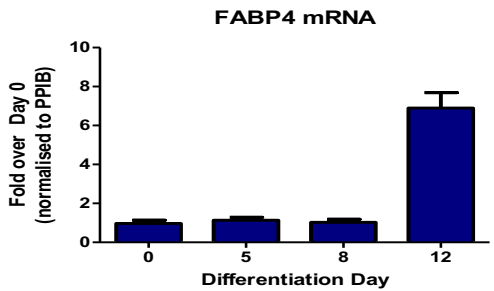


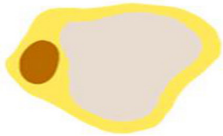




Pre-adipocytes
Present in the SVF of adipose tissue.
Secrete pro-inflammatory mediators such as IL-6 and MCP-1.
Adipocyte precursor.
A pro-inflammatory environment hampers adipogenesis.

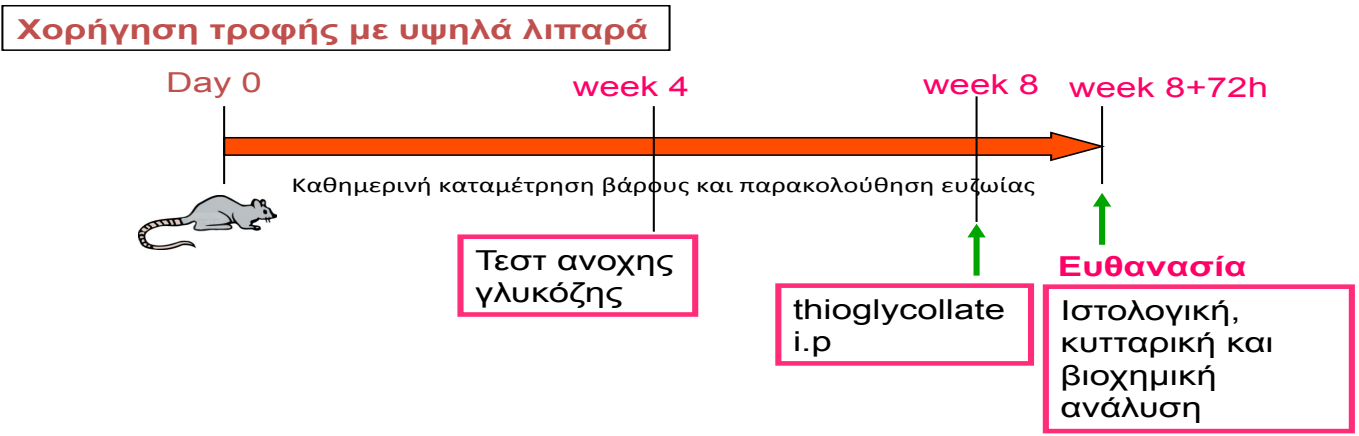






Adipocytes
Predominant cell type in adipose tissue.
Secretes adipokines including IL-6, leptin and adiponectin.
Release FFAs.

Επίδραση διατροφικών συμπληρωμάτων στη μεταβολική φλεγμονή και το διαβήτη τύπου 2



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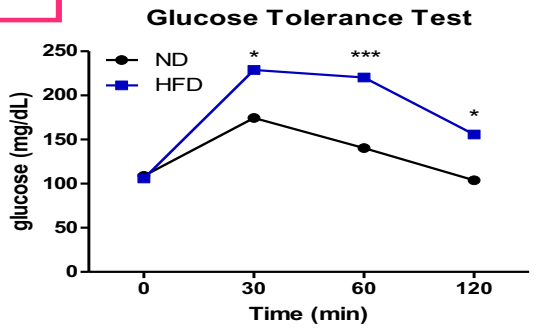
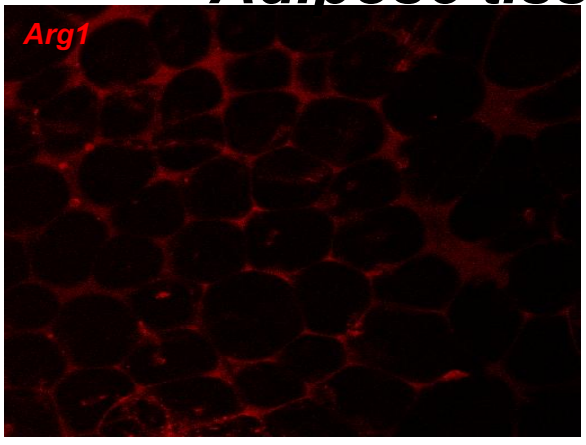
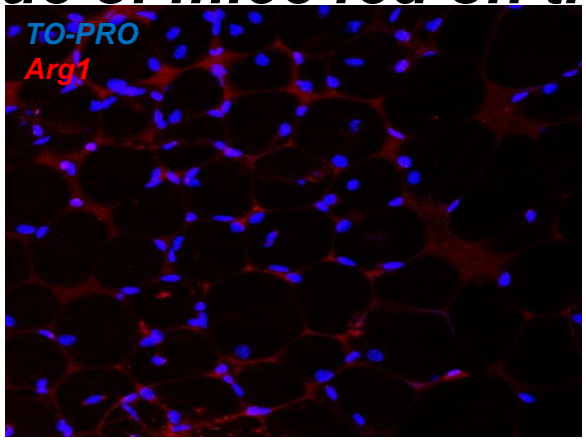
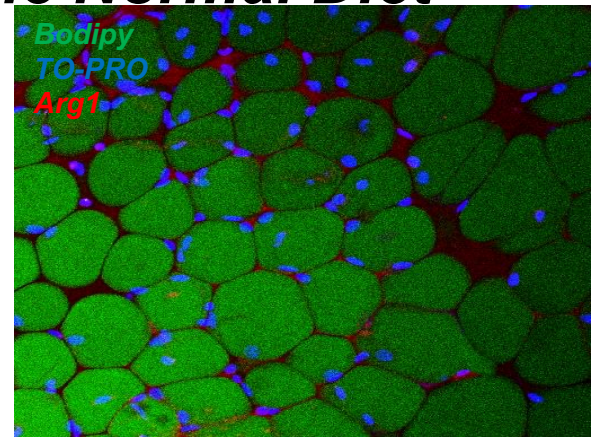


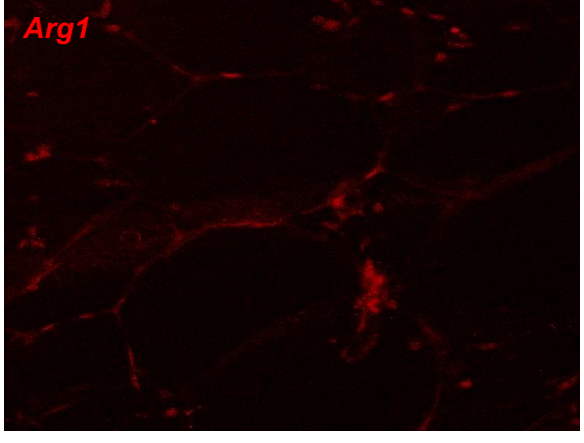
Adipose tissue of mice fed on the Normal Diet

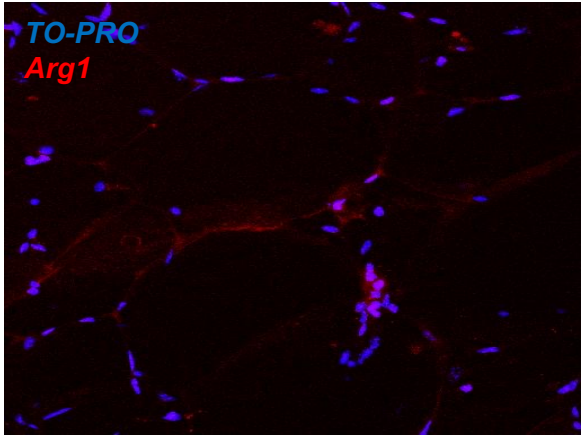


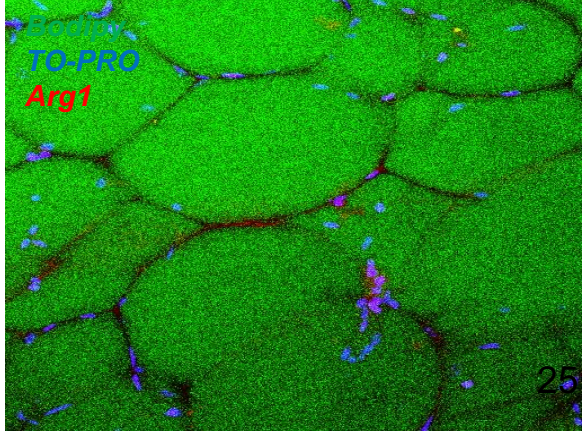




Adipose tissue of mice fed with High Fat Diet

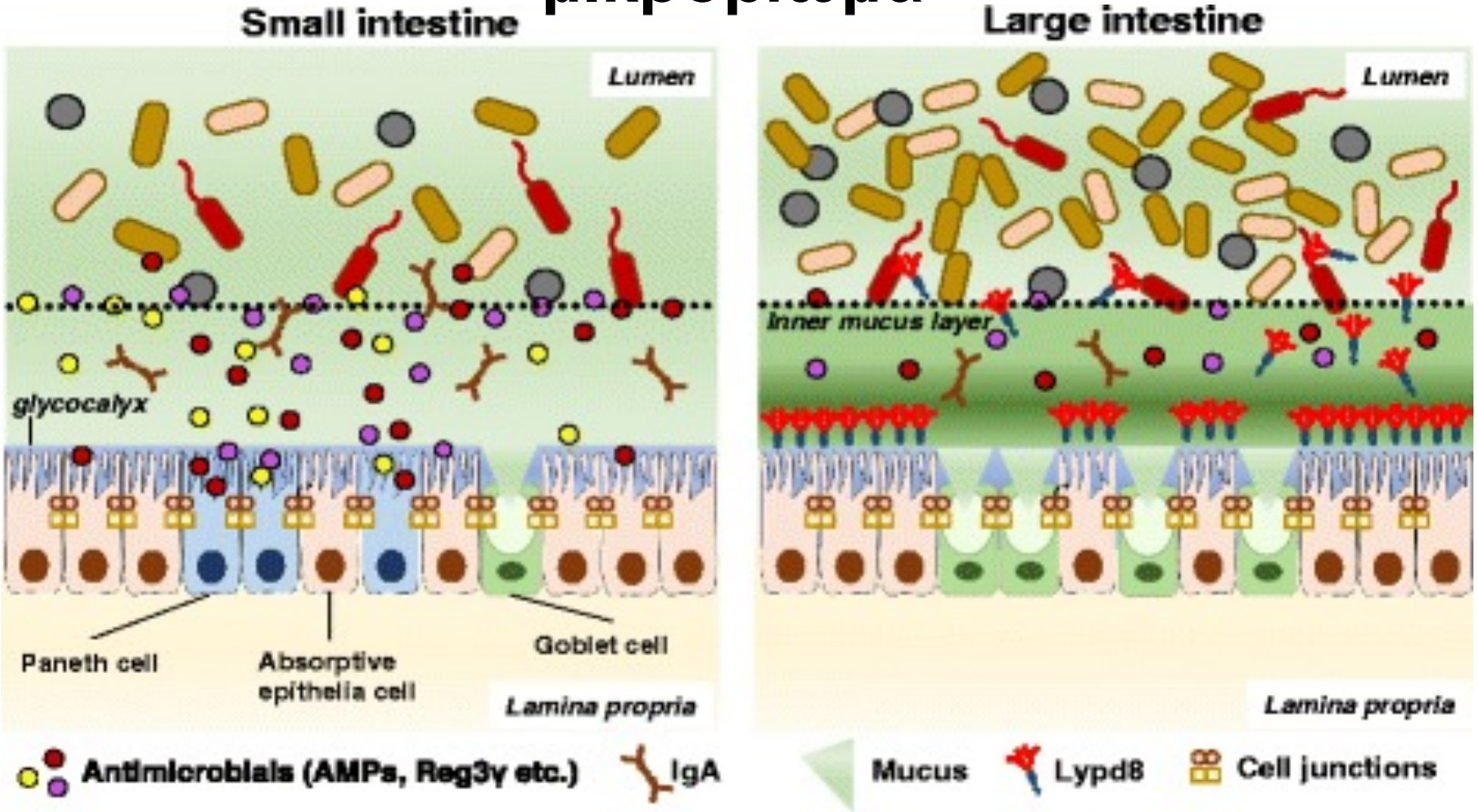


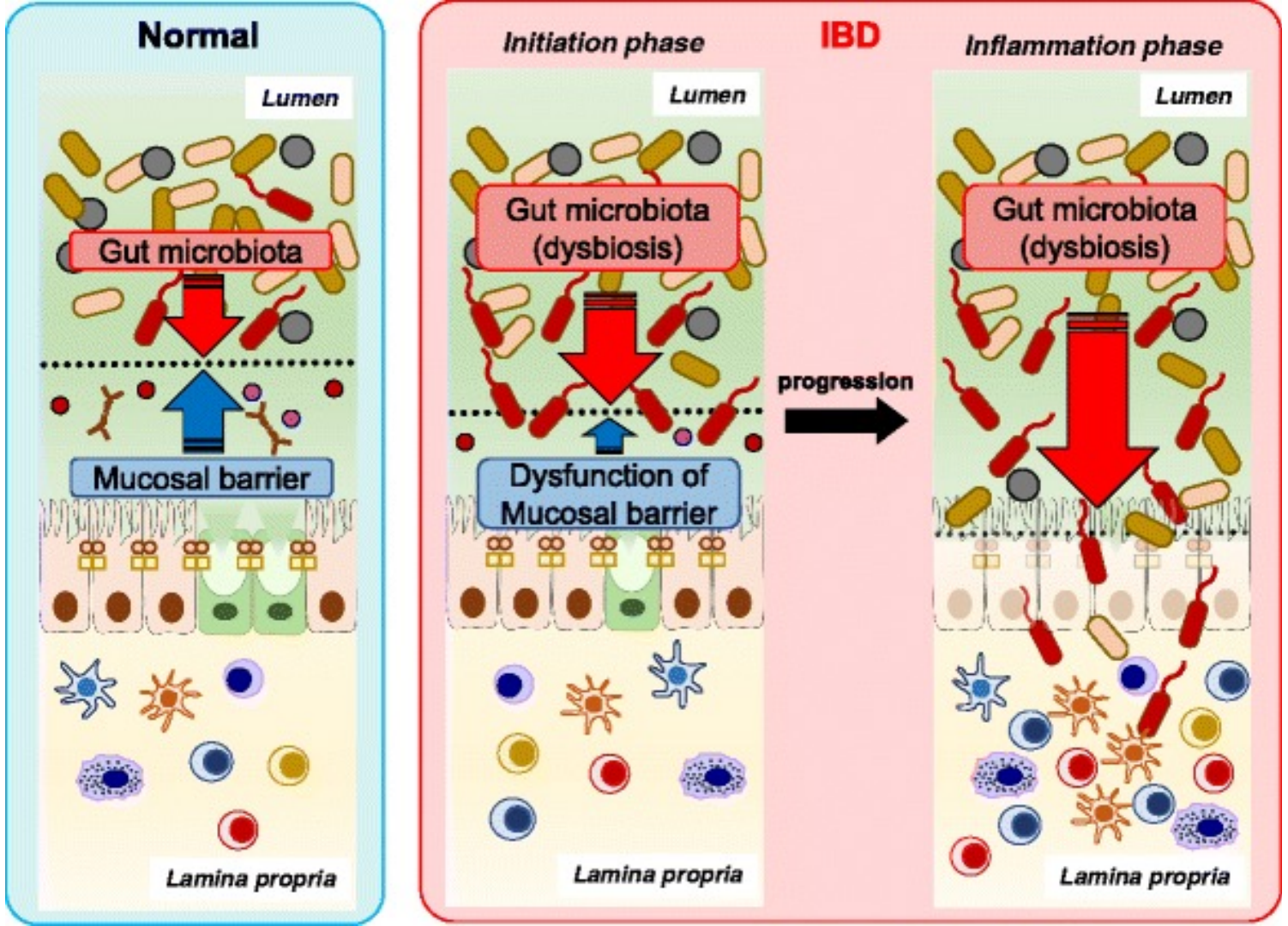




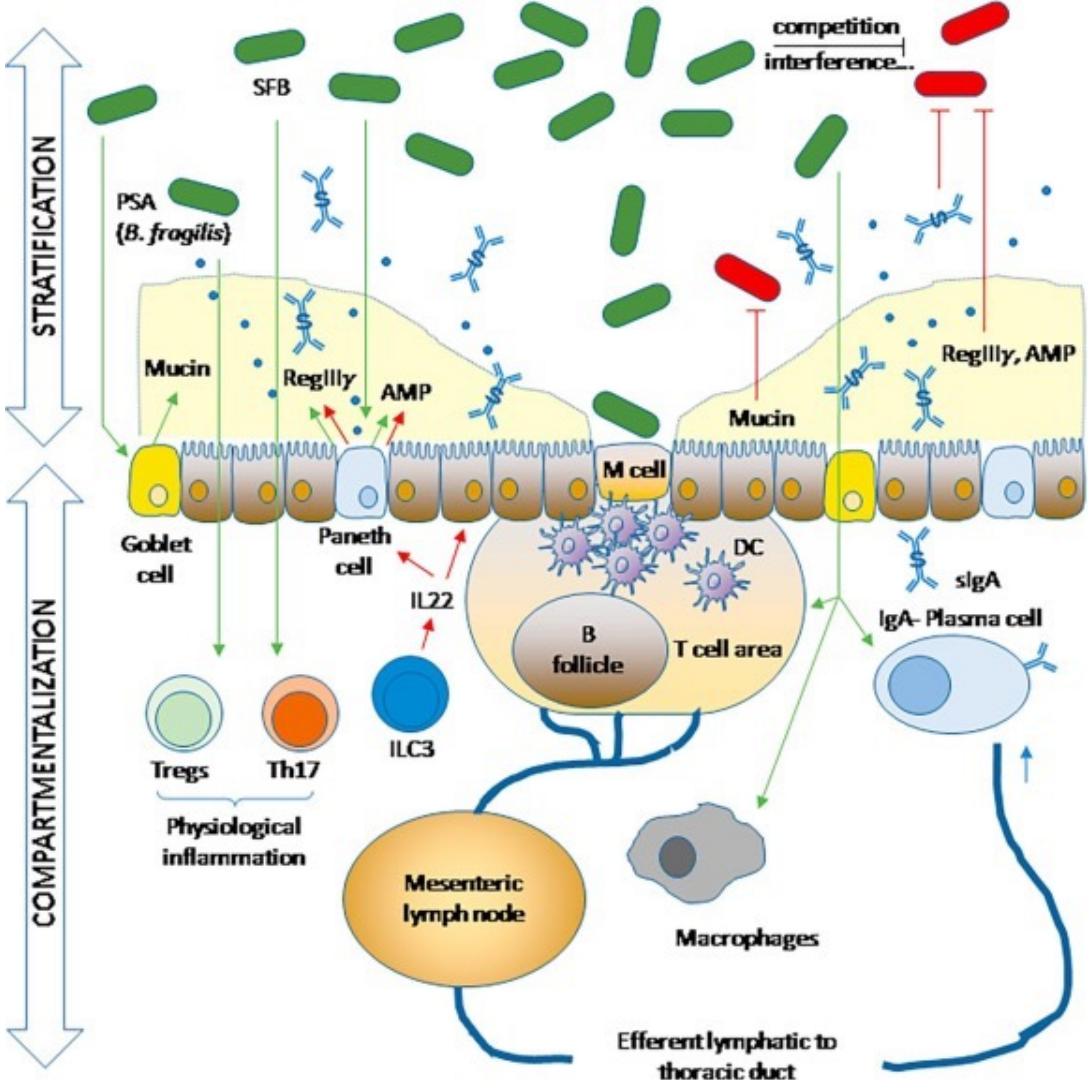
Ο ρόλος της διατροφής και διατροφικών συμπληρωμάτων στο εντερικό μικροβίωμα

Αλληλεπίδραση εντερικού επιθηλίου με το μικροβίωμα



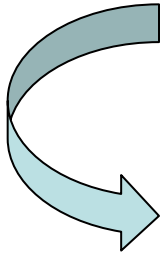

Normal
Lumen
Gut microbiota
Mucosal barrier
Lamina propria
IBD
Initiation phase
Lumen
Gut microbiota (dysbiosis)
Dysfunction of Mucosal barrier
Lamina propria
progression
Inflammation phase
Lumen
Gut microbiota (dysbiosis)
Lamina propria

Το ανοσοποιητικό αληλλεπιδρά με το μικροβίωμα

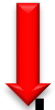


Diet alters gut flora

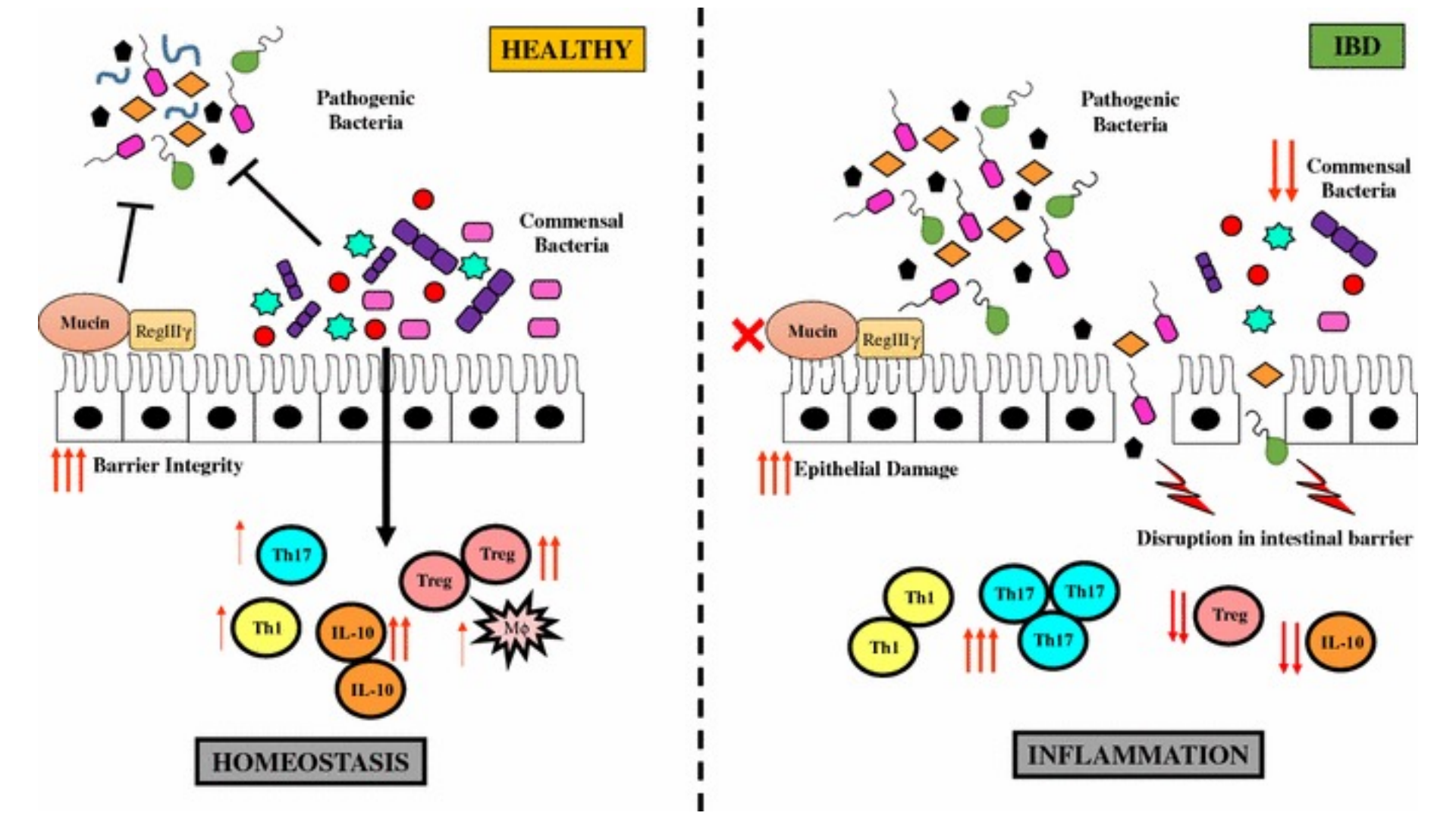
Altered gut flora affects inflammation

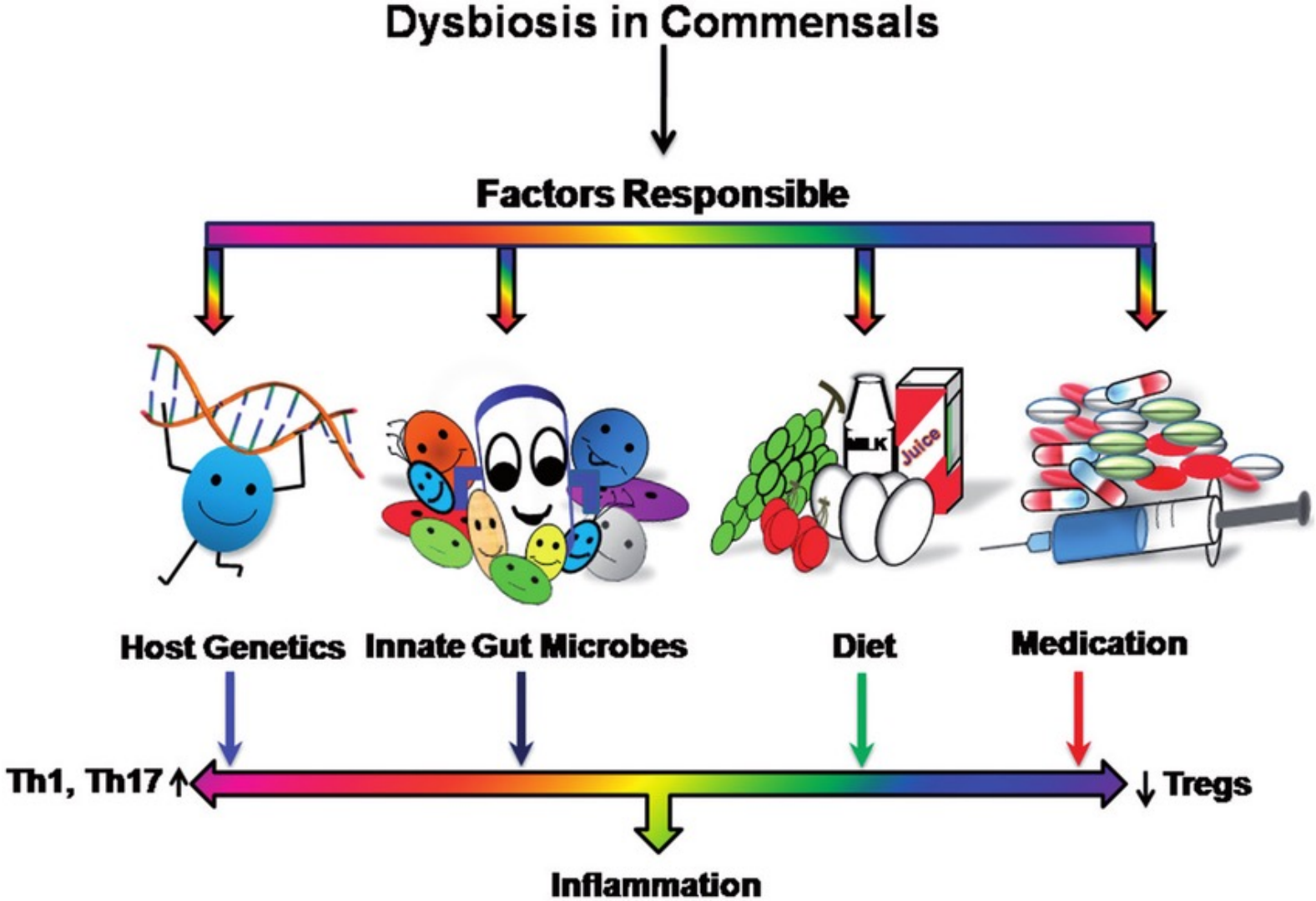


Inflammation alters gut flora

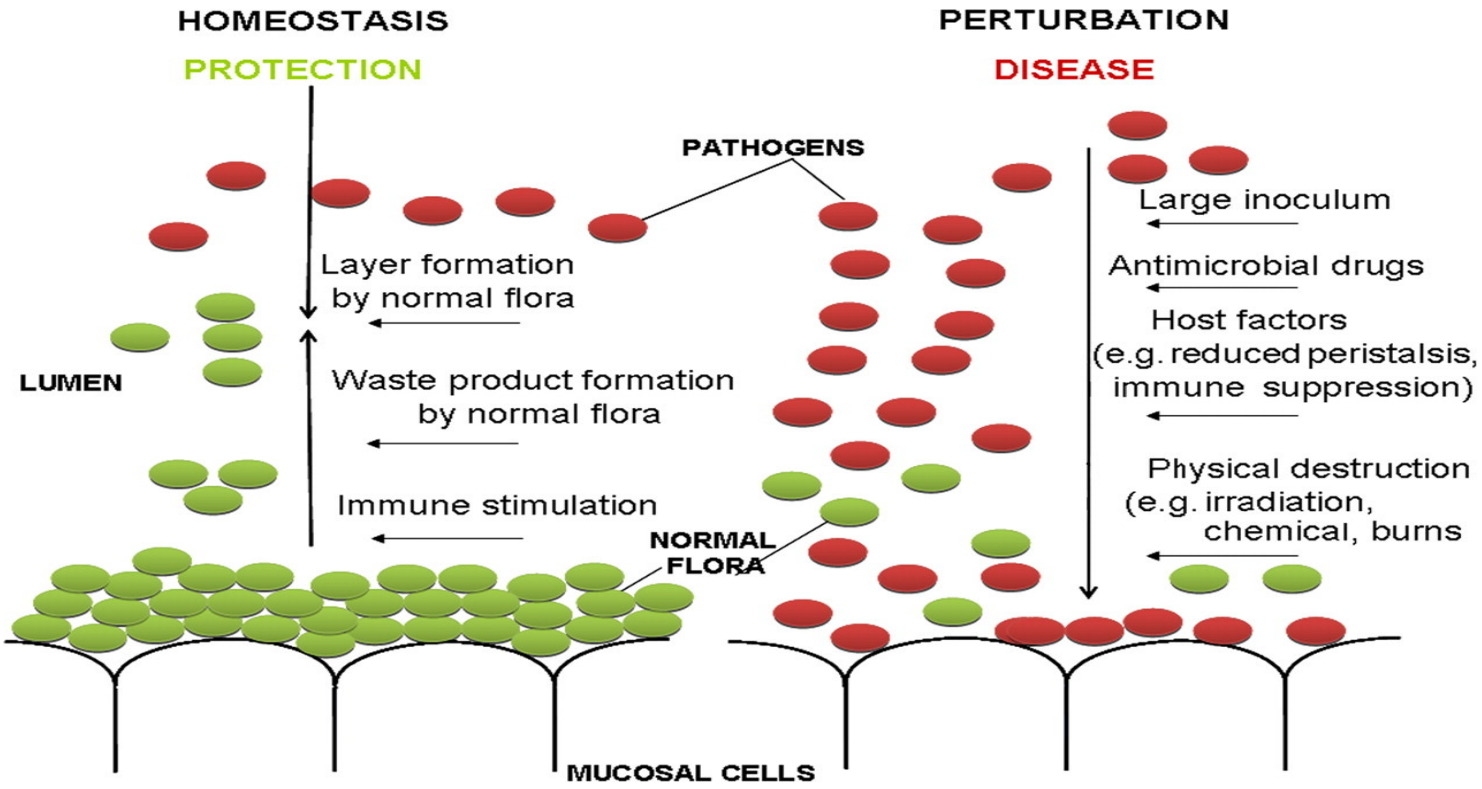


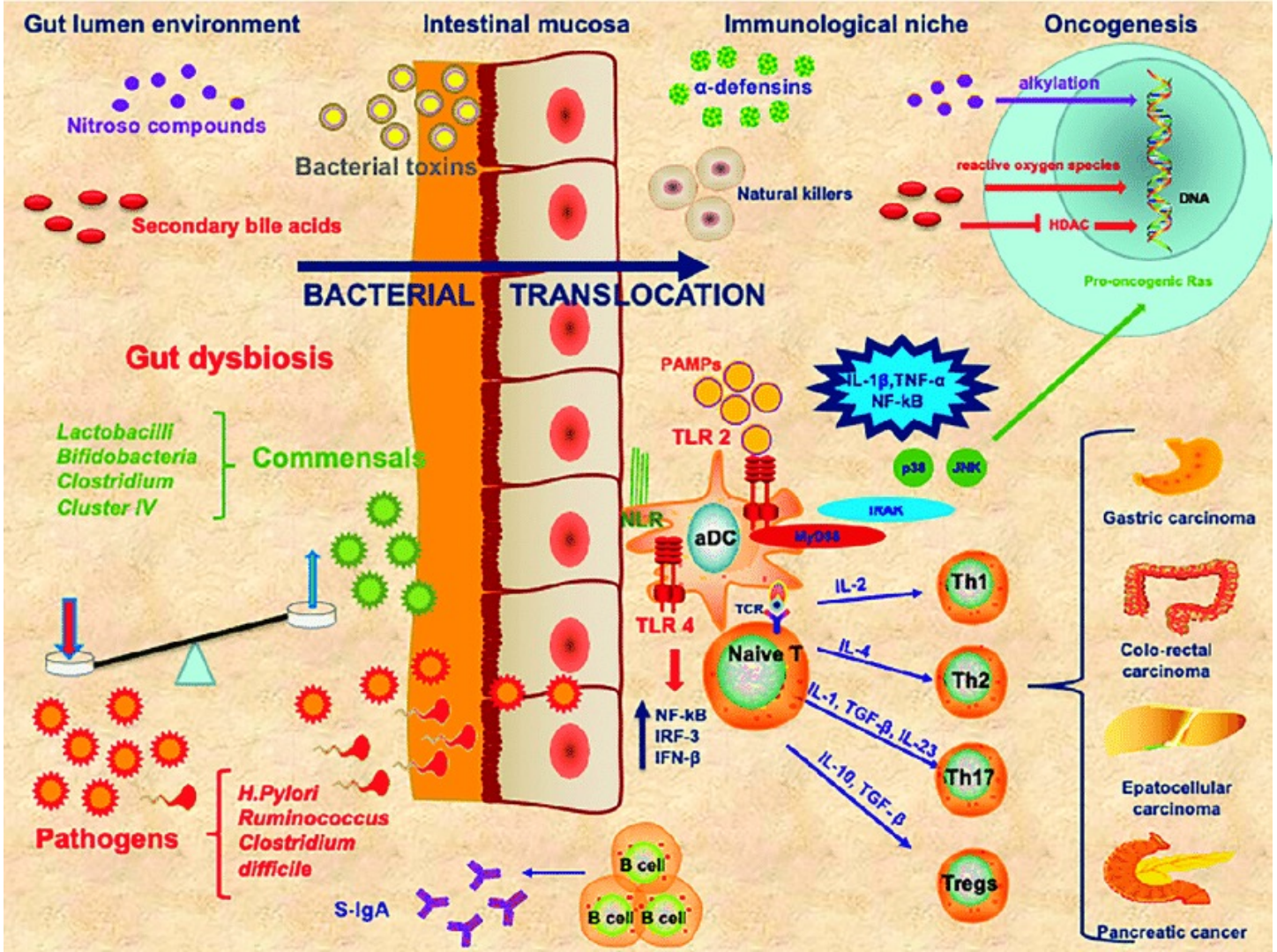
Obesity-induced inflammation is affected by the gut flora


HEALTHY
Pathogenic Bacteria
Commensal Bacteria
Mucin
RegIIIγ
Barrier Integrity
Th17
Treg
Treg
Th1
IL-10
Mϕ
IL-10
HOMEOSTASIS
IBD
Pathogenic Bacteria
Commensal Bacteria
Mucin
RegIIIγ
Epithelial Damage
Disruption in intestinal barrier
Th1
Th1
Th17
Th17
Th17
Treg
IL-10
INFLAMMATION


Dysbiosis in Commensals
Factors Responsible
Juice
Host Genetics
Innate Gut Microbes
Diet
Medication
Th1, Th17 ↑
↓ Tregs
Inflammation

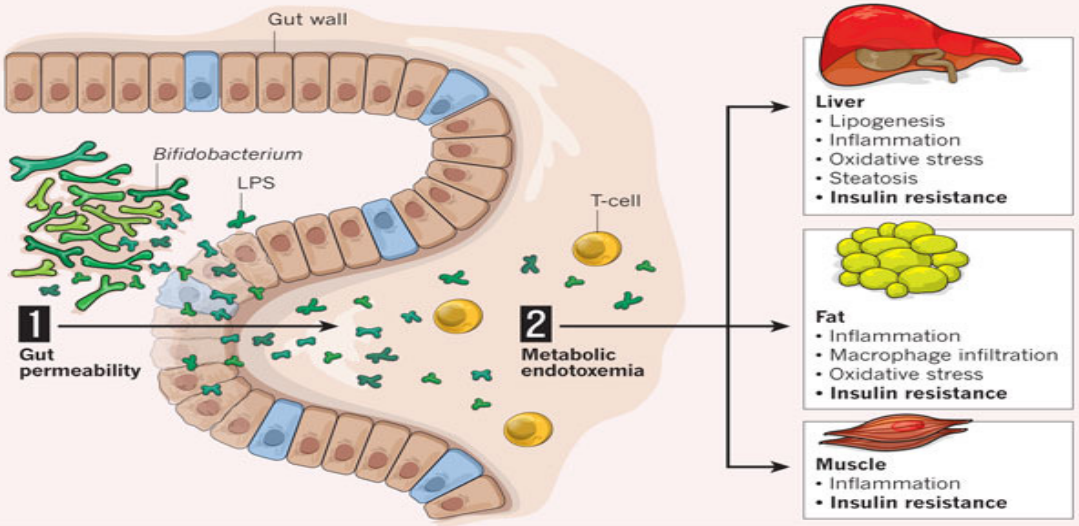
Function of gut flora



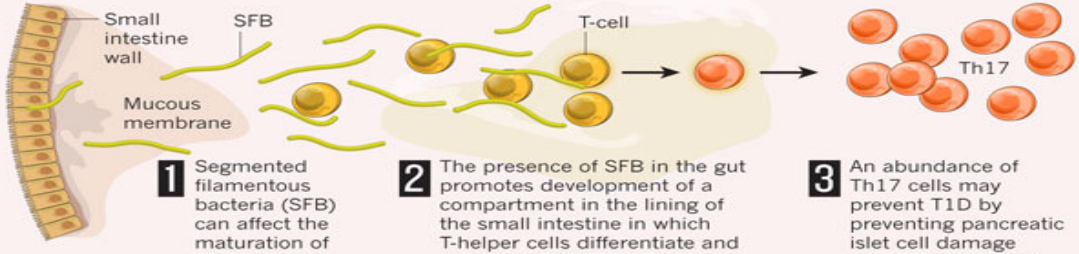

Gut lumen environment
Intestinal mucosa
Immunological niche
Oncogenesis
Nitroso compounds
Bacterial toxins
α-defensins
alkylation
reactive oxygen species
DNA
HDAC
Pro-oncogenic Ras
Natural killers
Secondary bile acids
BACTERIAL TRANSLOCATION
Gut dysbiosis
PAMPs
IL-1β,TNF-α
NF-kB
TLR 2
p38
JNK
Lactobacilli
Bifidobacteria
Clostridium
Cluster IV
Commensals
NLR
aDC
IRAK
MyD88
TLR 4
TCR
Naive T
IL-2
Th1
IL-4
Th2
IL-1, TGF-β, IL-23
Th17
IL-10, TGF-β
Tregs
NF-kB
IRF-3
IFN-β
Gastric carcinoma
Colo-rectal carcinoma
Epatocellular carcinoma
Pancreatic cancer
Pathogens
H.Pylori
Ruminococcus
Clostridium difficile
S-IgA
B cell
B cell
B cell

MICROBIAL INFLUENCE

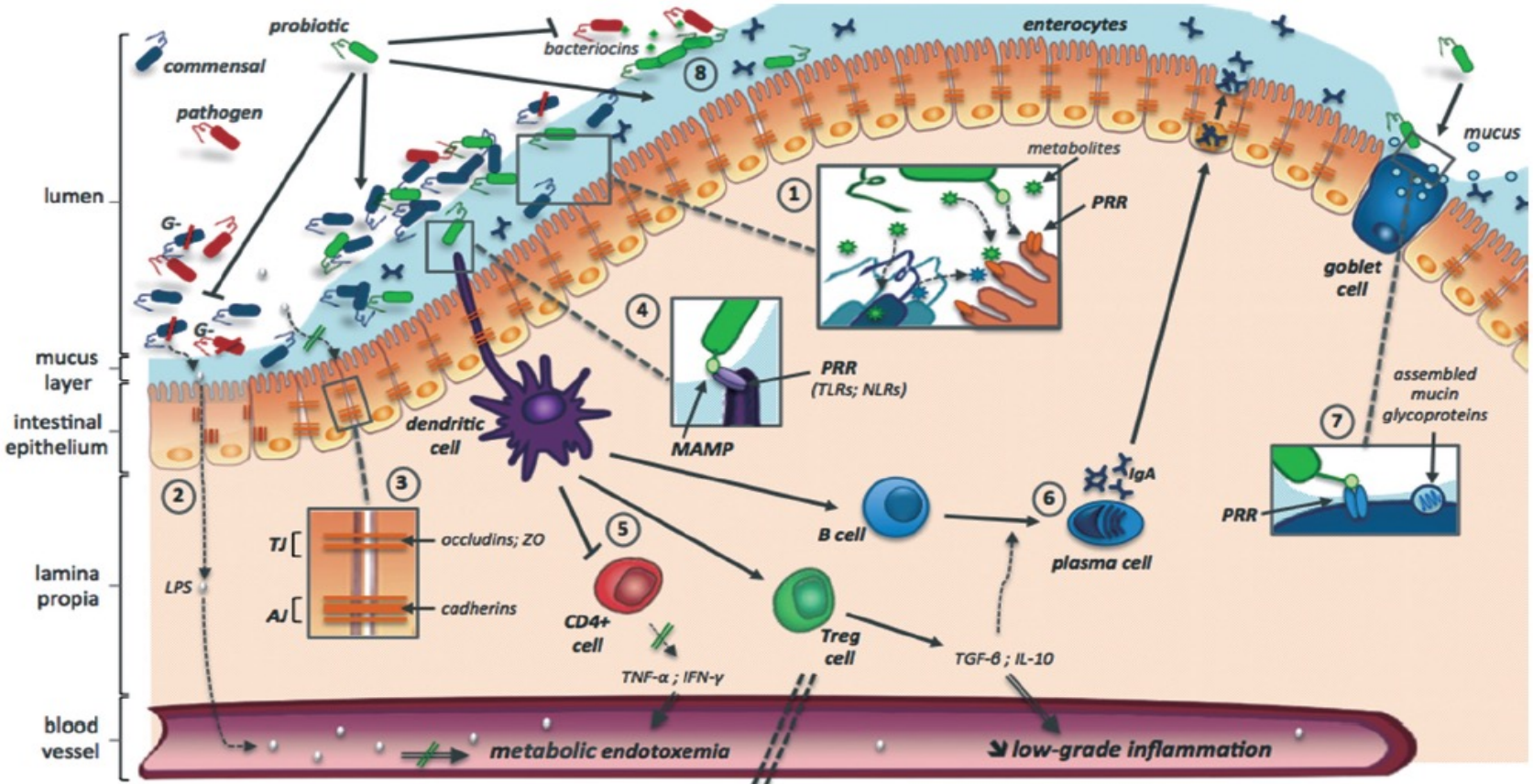
Research by Patrice Cani, at the Université Catholique de Louvain in Brussels, has shown that, in mice, a decrease in the population of bifidobacteria species in the gut causes the tight junctions between the cells of the gut lining to loosen. The loose junctions increase the gut's permeability and allow lipopolysaccharide (LPS) from these microbes to leak through the gut wall. The resulting metabolic endotoxaemia causes a low-grade inflammation and can induce a number of metabolic disorders – including the insulin resistance that characterizes T2D.

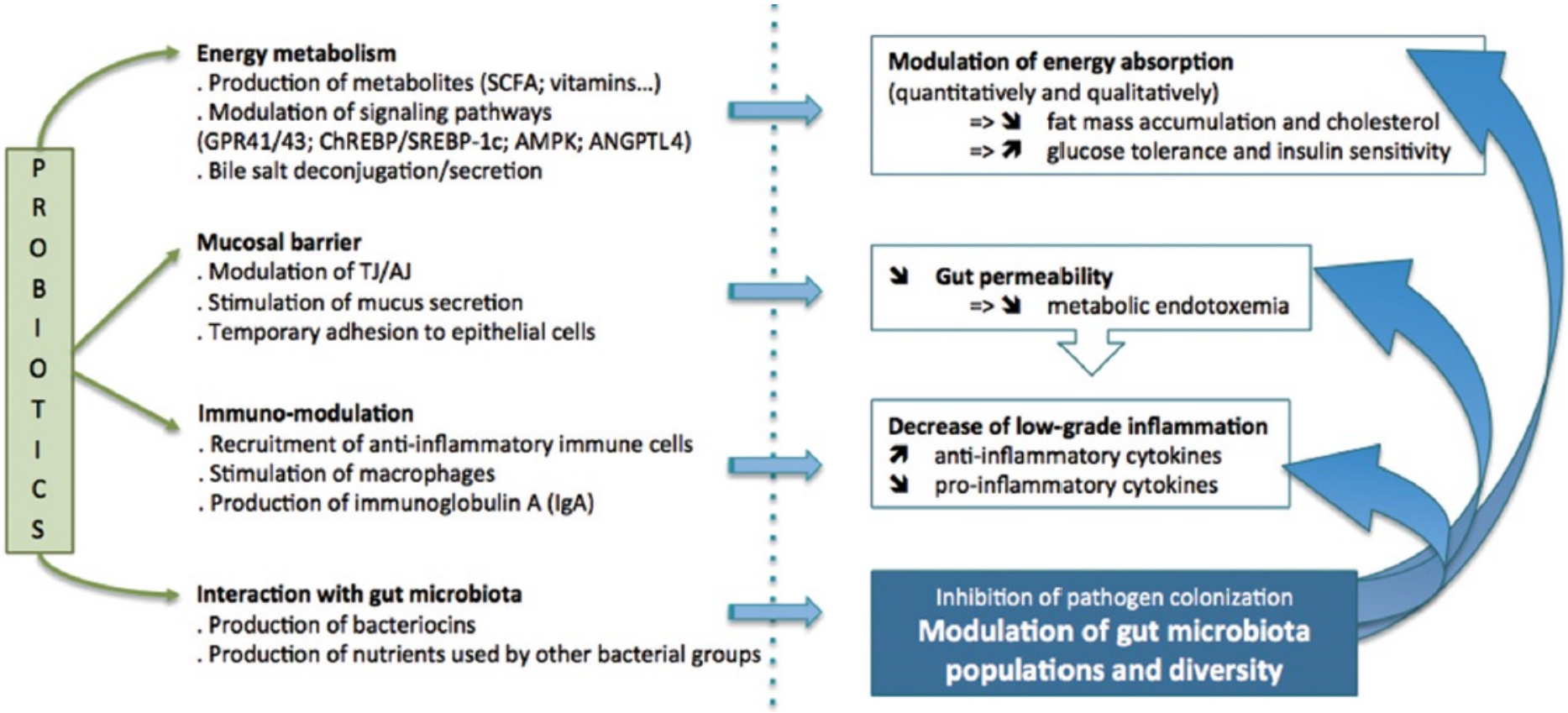


Research by Harvard immunologist Diane Mathis suggests that certain bacteria may protect against T1D.



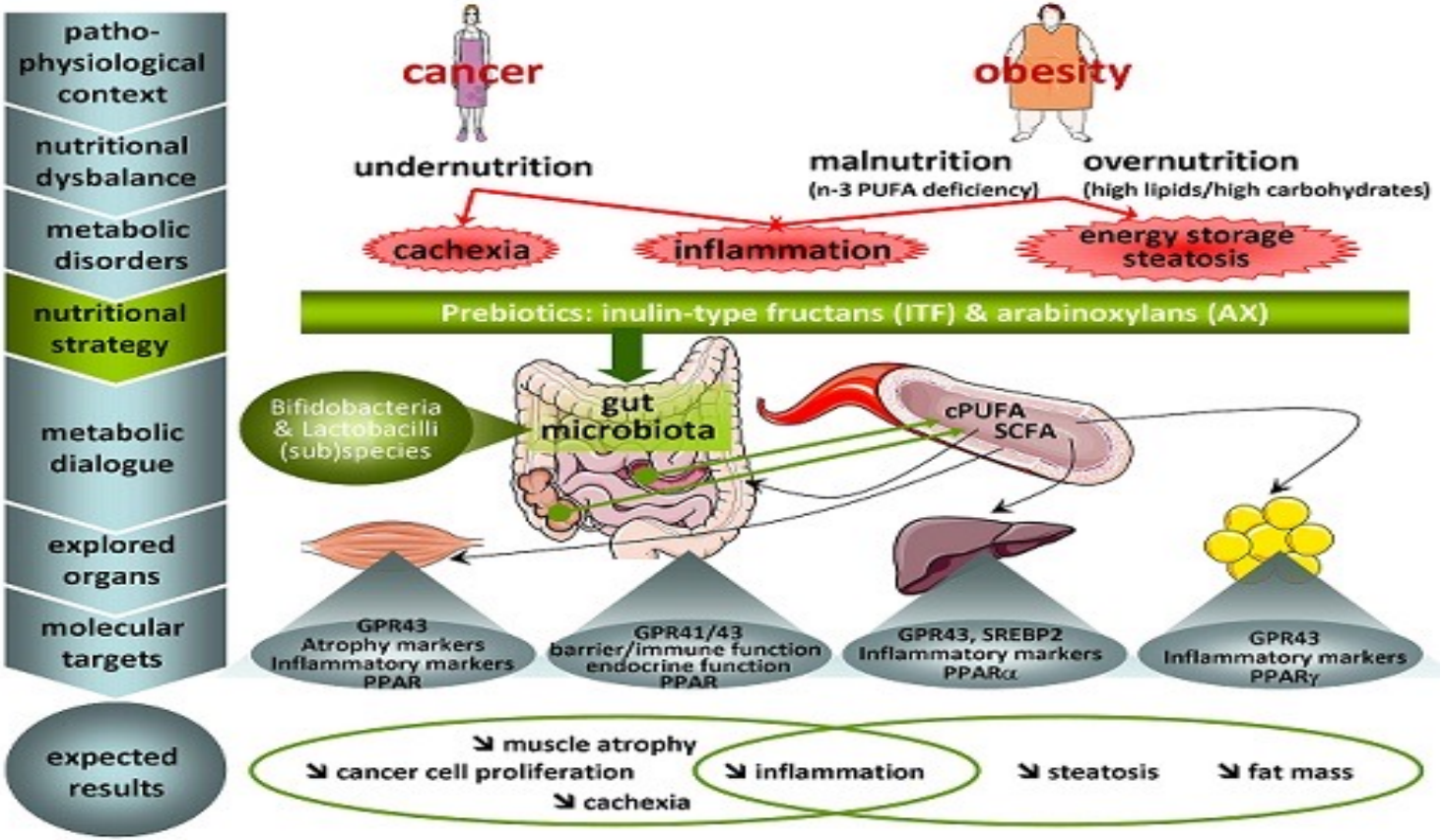
Probiotics support intestinal homeostasis



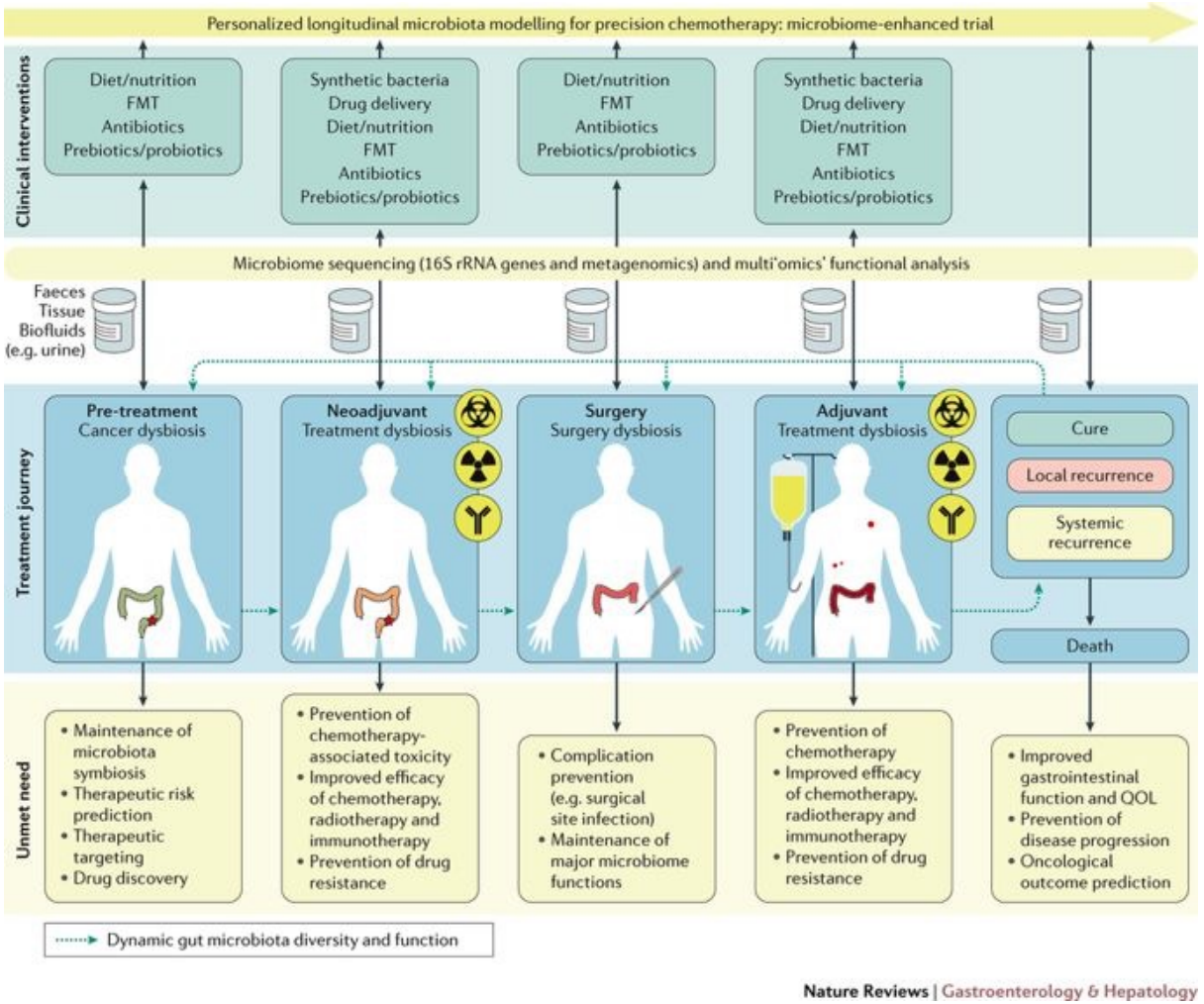

PROBIOTICS
Energy metabolism
. Production of metabolites (SCFA; vitamins...)
. Modulation of signaling pathways
(GPR41/43; ChREBP/SREBP-1c; AMPK; ANGPTL4)
. Bile salt deconjugation/secretion
Mucosal barrier
. Modulation of TJ/AJ
. Stimulation of mucus secretion
. Temporary adhesion to epithelial cells
Immuno-modulation
. Recruitment of anti-inflammatory immune cells
. Stimulation of macrophages
. Production of immunoglobulin A (IgA)
Interaction with gut microbiota
. Production of bacteriocins
. Production of nutrients used by other bacterial groups
Modulation of energy absorption
(quantitatively and qualitatively)
=> ↘ fat mass accumulation and cholesterol
=> ↗ glucose tolerance and insulin sensitivity
↘ Gut permeability
=> ↘ metabolic endotoxemia
Decrease of low-grade inflammation
↗ anti-inflammatory cytokines
↘ pro-inflammatory cytokines
Inhibition of pathogen colonization
Modulation of gut microbiota populations and diversity

Disease affects gut microbiome

Obesity-Cancer-Gut flora



Therapy promotes dysbiosis: potential treatments



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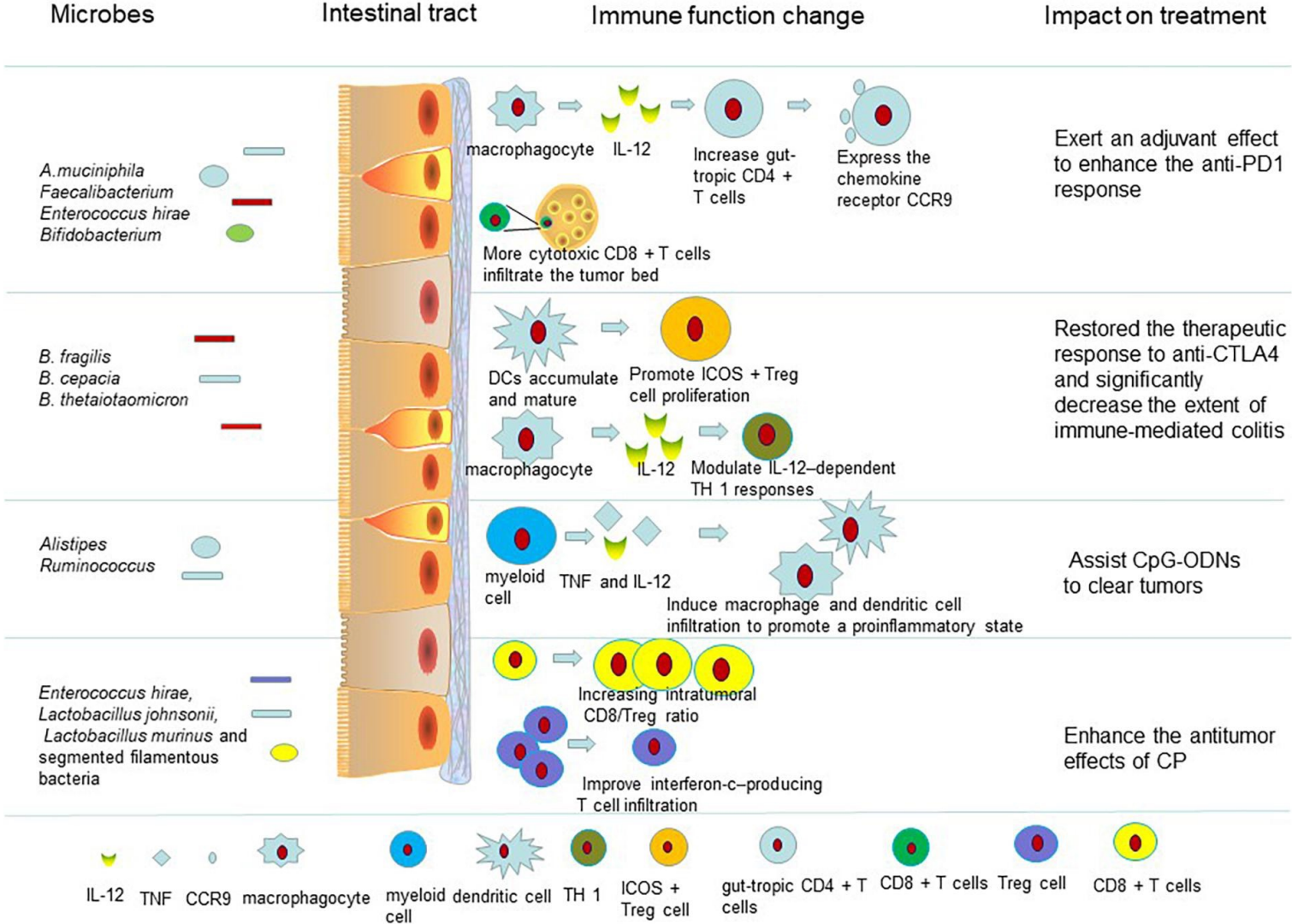
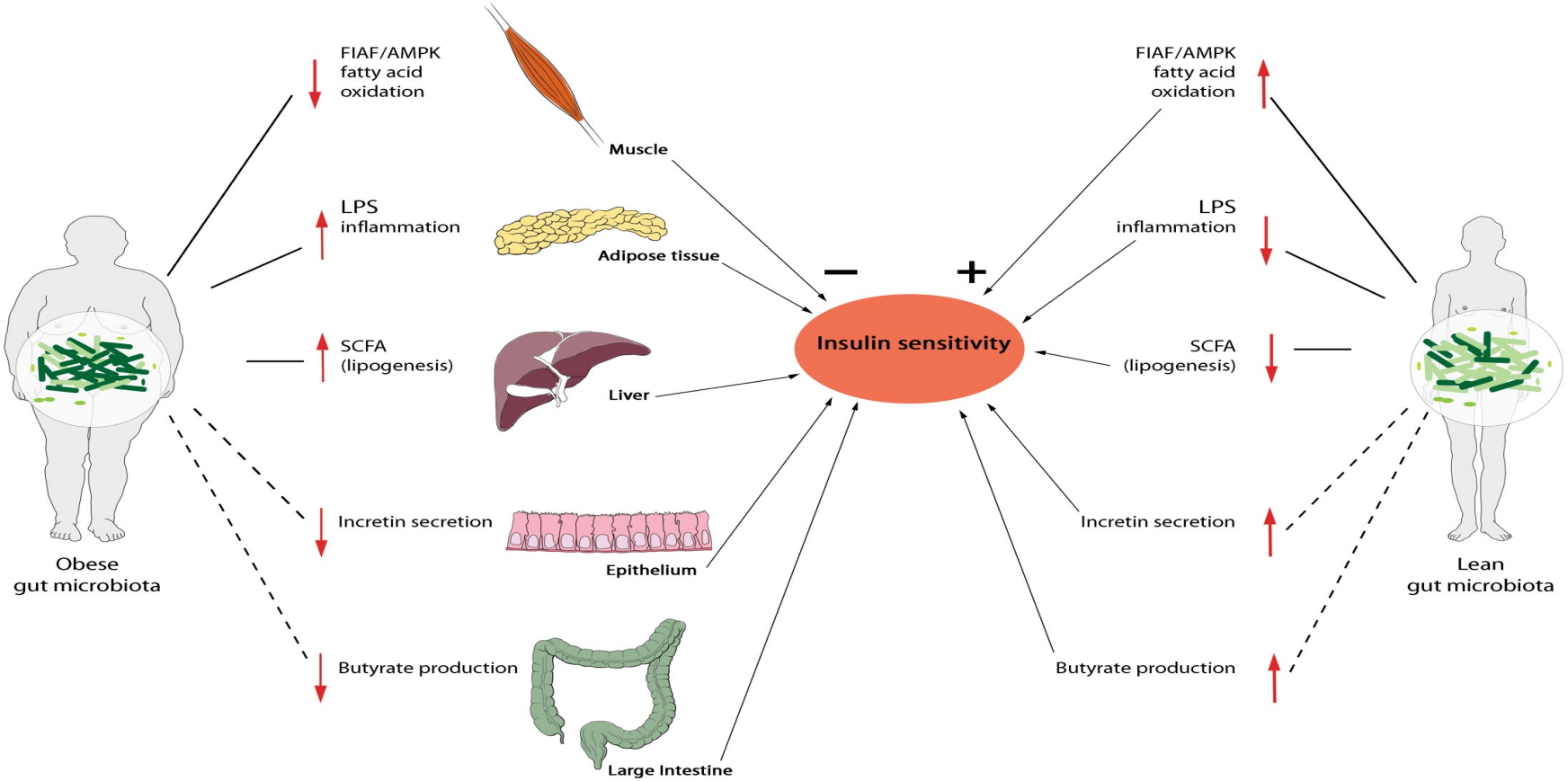
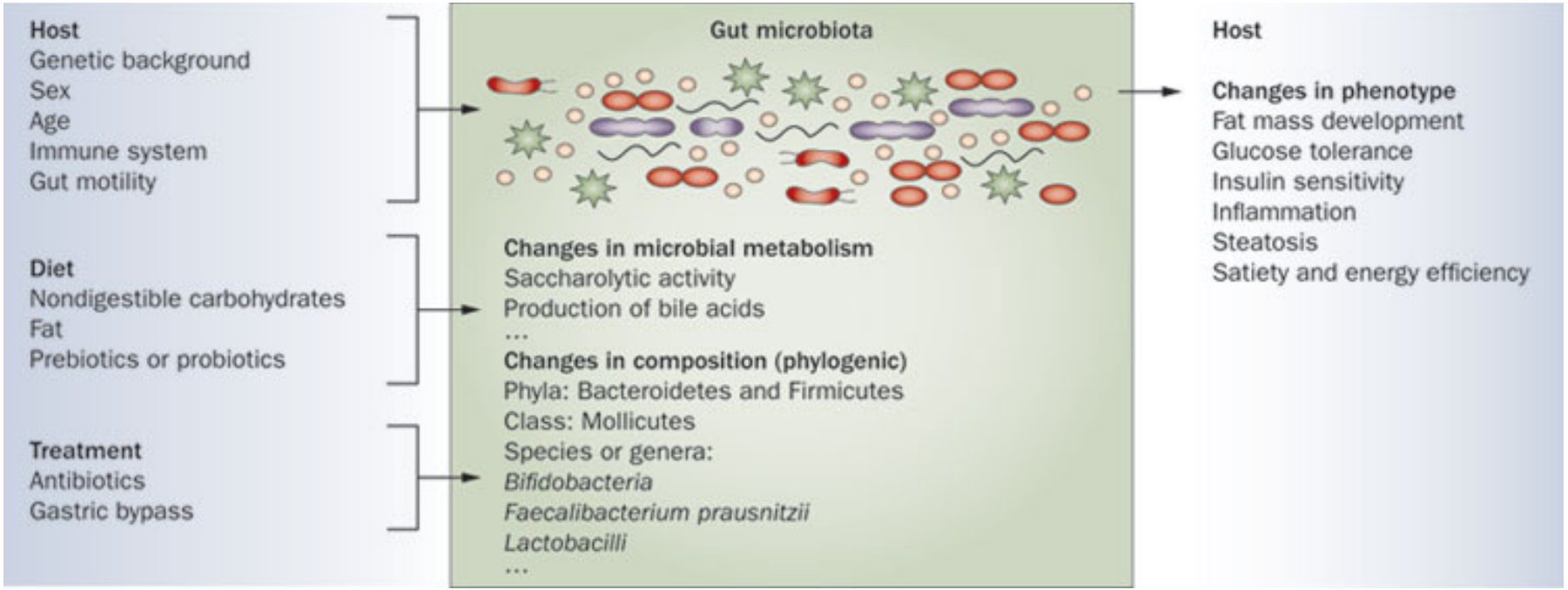
Cancer immunotherapy is improved with a healthy gut microbiome


Microbes
Intestinal tract
Immune function change
Impact on treatment
A.muciniphila
Faecalibacterium
Enterococcus hirae
Bifidobacterium
macrophagocyte
IL-12
Increase gut-tropic CD4 + T cells
Express the chemokine receptor CCR9
More cytotoxic CD8 + T cells infiltrate the tumor bed
Exert an adjuvant effect to enhance the anti-PD1 response
B. fragilis
B. cepacia
B. thetaiotaomicron
DCs accumulate and mature
Promote ICOS + Treg cell proliferation
macrophagocyte
IL-12
Modulate IL-12–dependent TH 1 responses
Restored the therapeutic response to anti-CTLA4 and significantly decrease the extent of immune-mediated colitis
Alistipes
Ruminococcus
myeloid cell
TNF and IL-12
Induce macrophage and dendritic cell infiltration to promote a proinflammatory state
Assist CpG-ODNs to clear tumors
Enterococcus hirae,
Lactobacillus johnsonii,
Lactobacillus murinus and segmented filamentous bacteria
Increasing intratumoral CD8/Treg ratio
Improve interferon-c–producing T cell infiltration
Enhance the antitumor effects of CP
IL-12
TNF
CCR9
macrophagocyte
myeloid cell
dendritic cell
TH 1
ICOS + Treg cell
gut-tropic CD4 + T cells
CD8 + T cells
Treg cell
CD8 + T cells

Gut microbiome affects insulin sensitivity



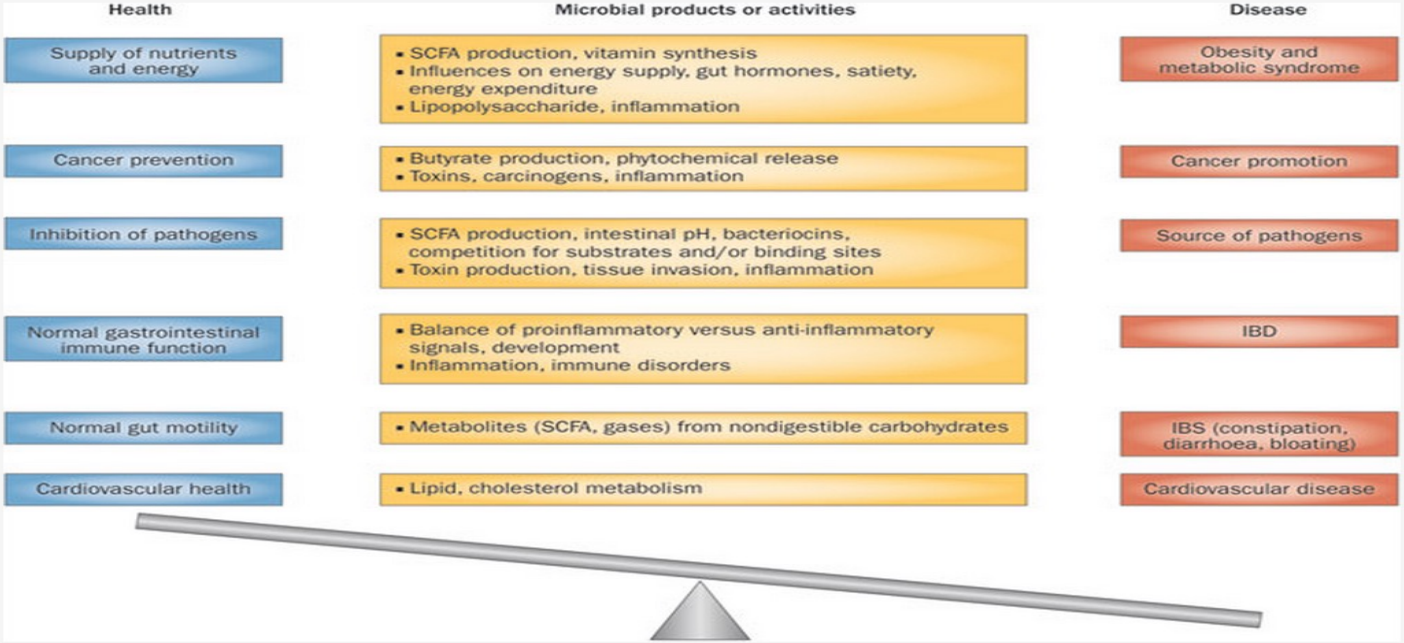
Parameters affecting gut microflora



Effects of gut microbiota in health and disease

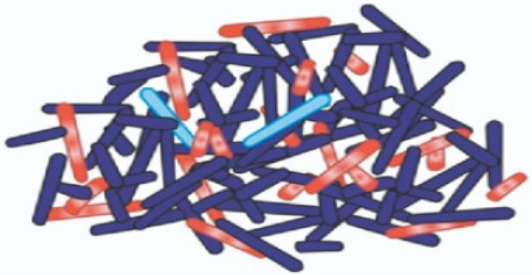
Harry J. Flint, Karen P. Scott, Petra Louis & Sylvia H. Duncan

Nature Reviews Gastroenterology & Hepatology **9**, 577-589 (October 2012)

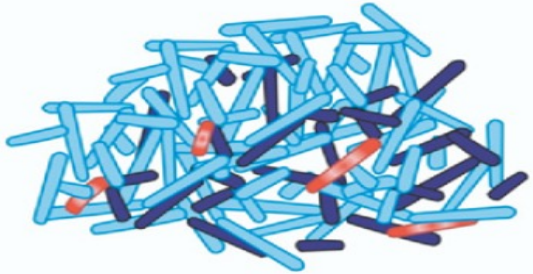


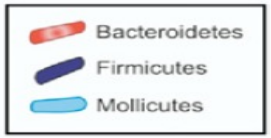
Diet alters gut microbiota

Low-fat, high-polysaccharide (CHO) diet



High-fat, high-sugar (Western) diet



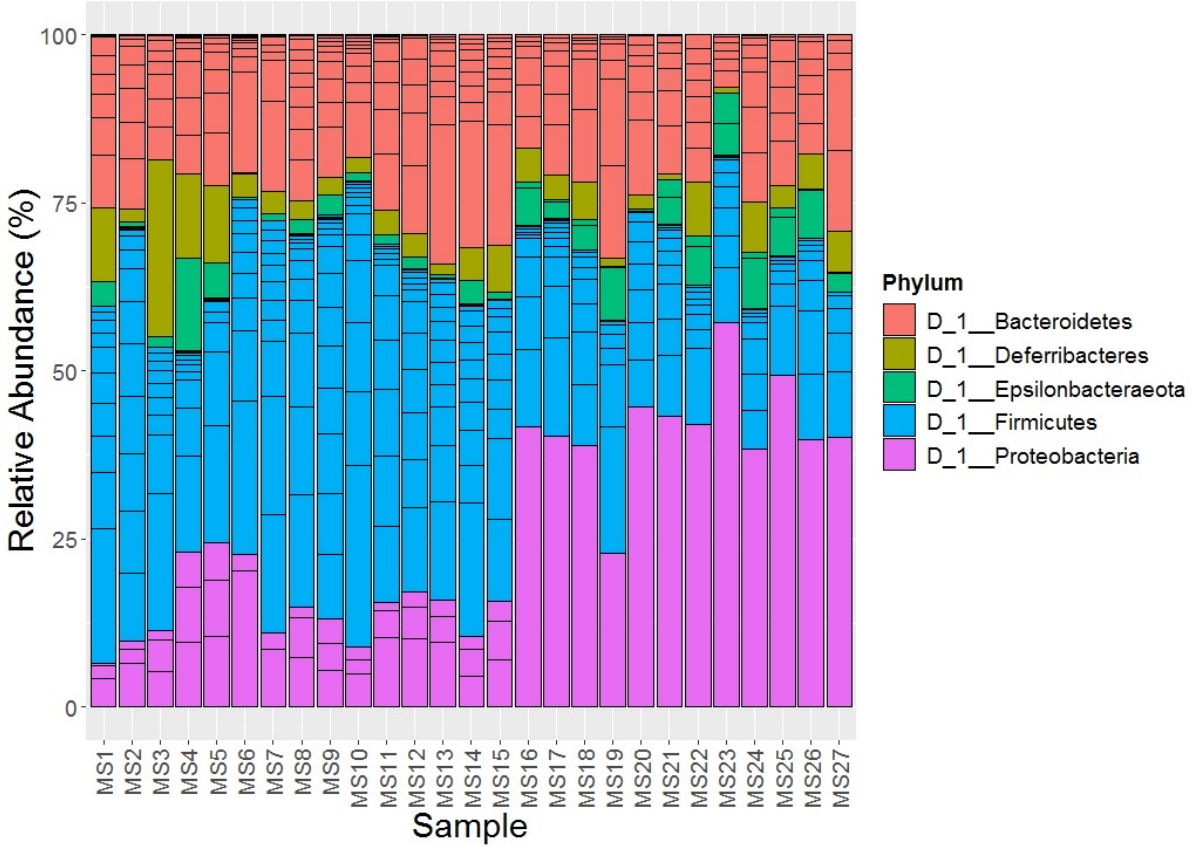


Changes in gut microbial ecology:
- reduction in Bacteroidetes and proportional increase in Firmicutes
- dramatic fall of overall diversity
- bloom of a single class of Firmicutes: the Mollicutes

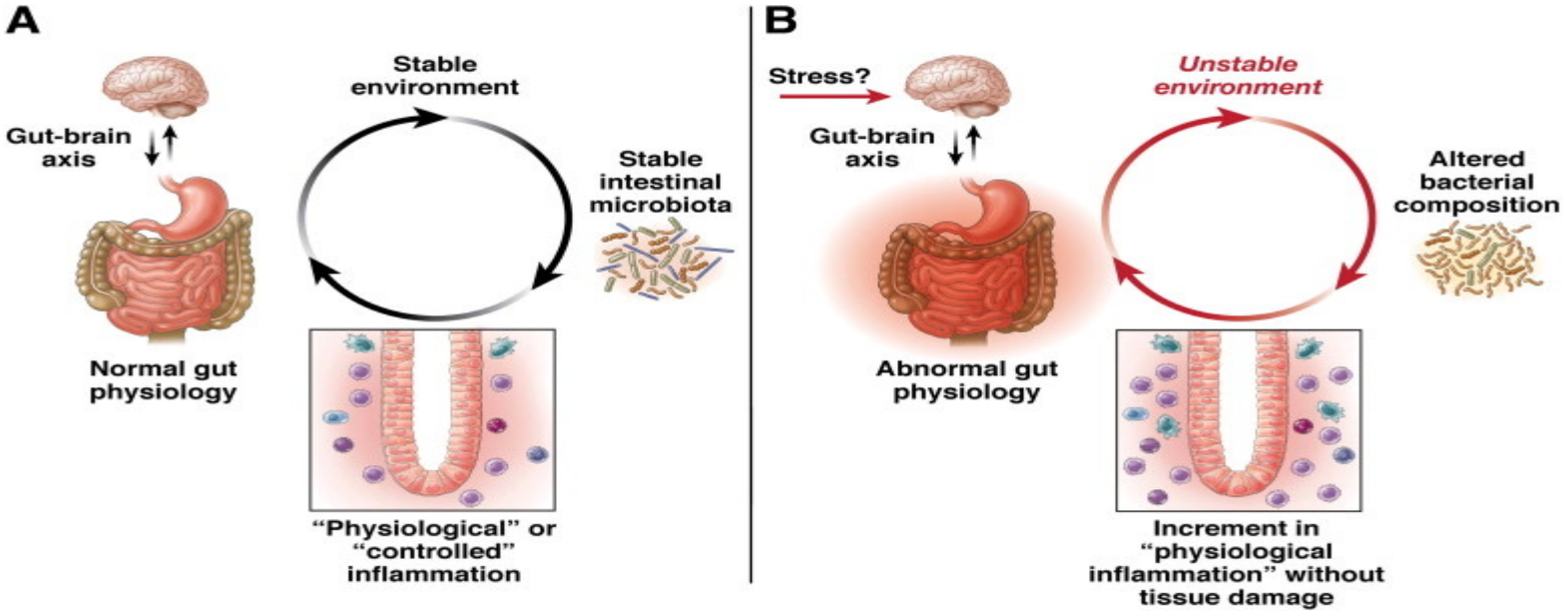
Alterations of metabolic potential:
- enrichment for phosphotransferase systems: import and fermentation of simple sugars
- enrichment for genes encoding beta-fructosidases
- depletion for motility genes, e.g. bacterial chemotaxis, motility proteins, flagellar assembly

Consequences:
- increased capacity to import "Western-diet"-typical carbohydrates
- increased capacity to metabolize imported sugars to short-chain fatty acids

Effect of diet supplementation on microbiome Phyla

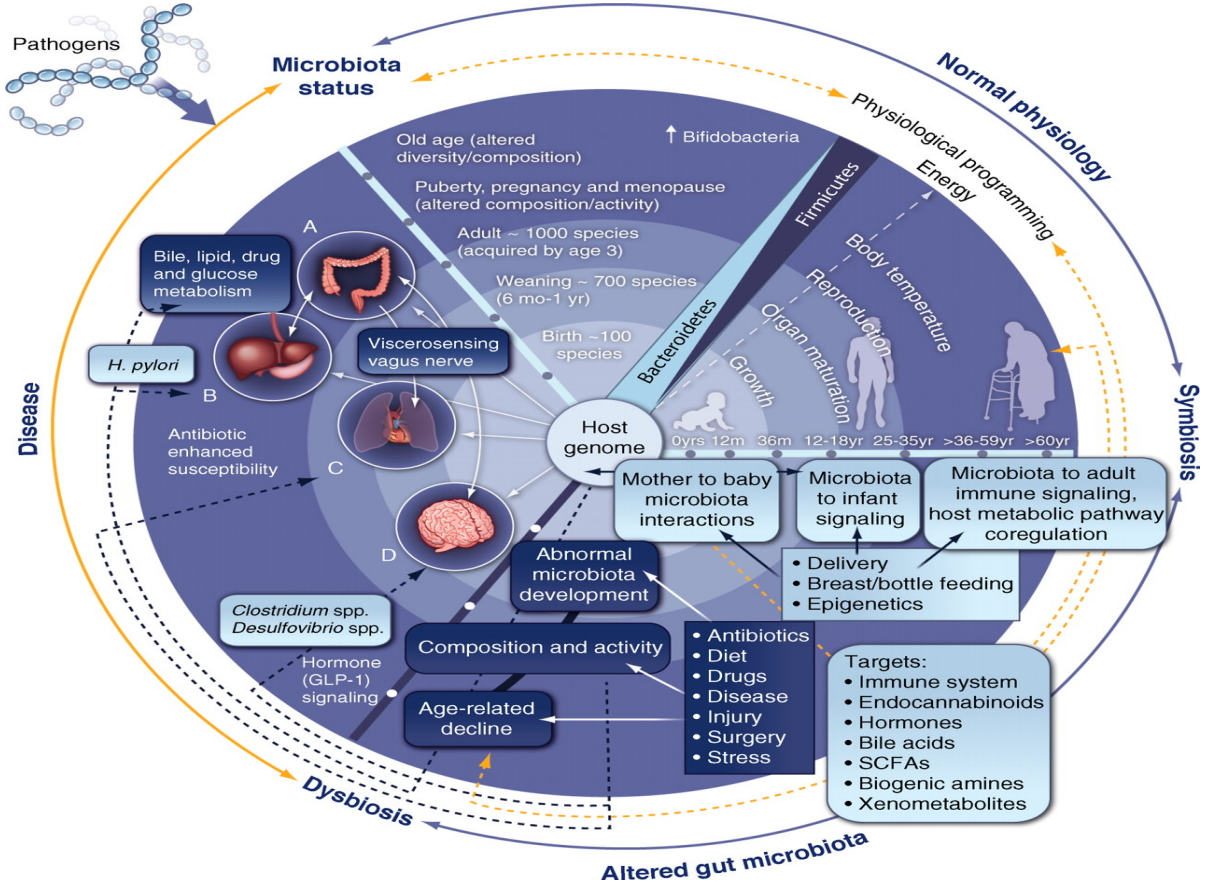


Gut-Brain axis



Neuropeptides posess anti-microbial activity

Gut microbiota and disease



Συμπεράσματα

- Το μικροβίωμα επηρεάζεται απο το ανοσοποιητικό και το ανοσοποιητικό απο το μικροβίωμα
- Η διατροφή επηρεάζει άμεσα το μικροβίωμα
- Το μικροβίωμα αλληλεπιδρά με νόσους και συνεισφέρει στη θεραπεία