

The Bile Acid Biome and its Relevance for NAFLD and Oncogenesis

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Outline of Presentation

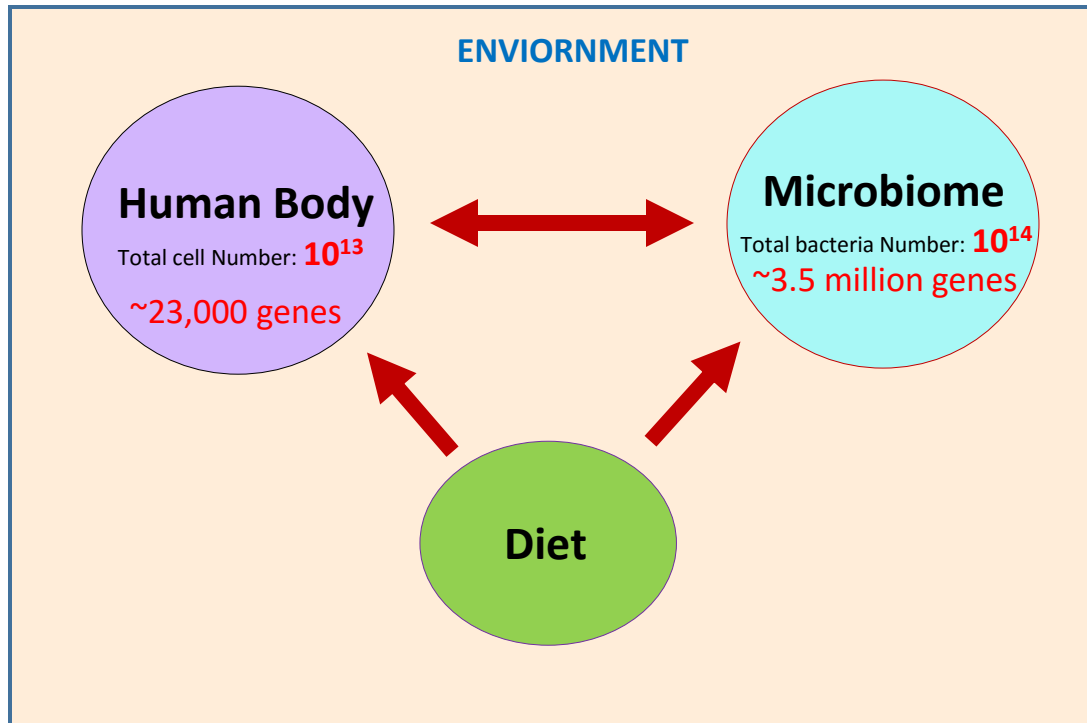
I. Introduction

II. Gut microbiome

III. Metabolism of bile acids by gut microbiota

- a) Bile acid 7 α -dehydroxylation
- b) Bile acid 7 α -dehydratase
- IV. Regulation of bile acid pool composition by gut bacteria
 - a) Implication for bile acid cell signaling
- V. Deoxycholic acid and liver cancer
- VI. Summary

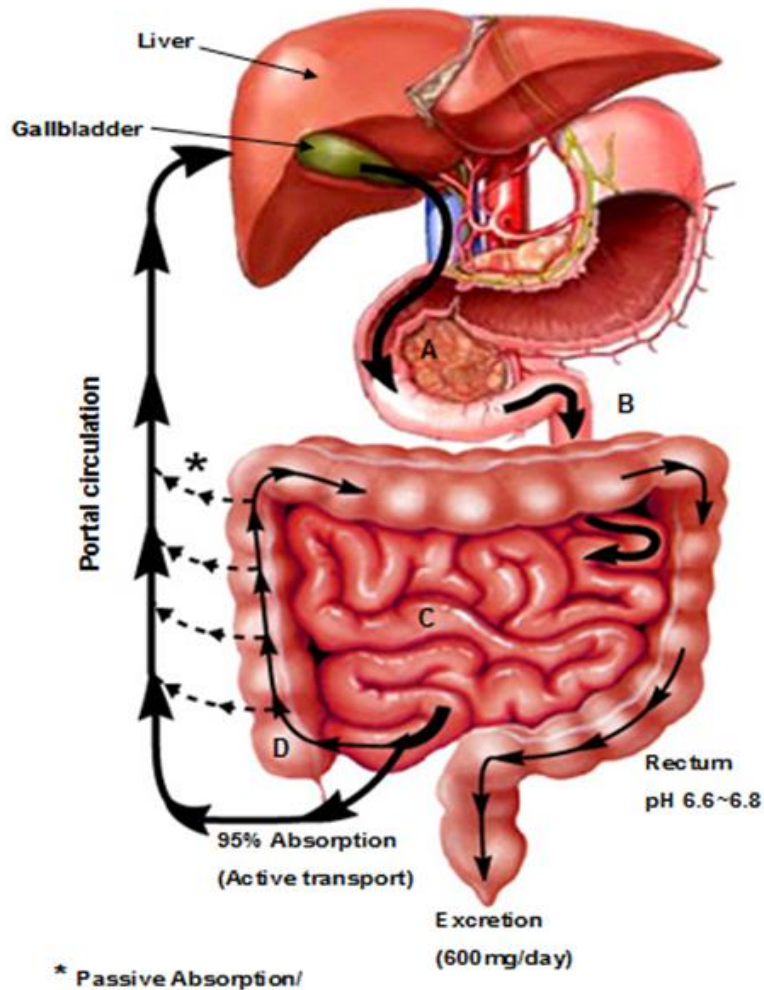
The Human Body as an Ecosystem



The Human Body is a Complex Ecosystem: Cells in the Body and Microbiome Interact; Both are Regulated by Diet

- The adult human body contains 10 trillion mammalian cells and 100 trillion bacterial cells and most are in the GI tract.
- The human gut contains ~2,000 bacterial species and >99% are obligate anaerobic bacteria.
- The combined genomes of gut bacteria encodes 3.5 million non-redundant genes and is ~143-times greater than the mammalian genome.
- The lumen of the GI tract is a highly anaerobic environment.
- Gut bacteria can carry out hundreds of enzymatic reactions that host cells can not catalyze.
- Approx. 35% of all blood metabolites are derived from gut bacterial metabolism.

Bacteria Levels in the Gastrointestinal Tract



Small Intestine

A: Duodenum (25cm) pH 5.7~6.4

$\sim 10^3$ Bacteria/ml

Lactobacillus spp. Streptococcus spp.

B: Jejunum (1.0m) pH 5.9~6.8

$\sim 10^4$ Bacteria/ml

Lactobacillus spp. Streptococcus spp.

Staphylococcus spp. Veillonella spp.

C: Ileum (2.0m) pH 7.3~7.7

$10^6 \sim 10^8$ Bacteria/ml

Enterobacteria, Enterococcus spp.

Bacteroides, Clostridia

Lactobacillus spp. Veillonella spp.

Large Intestine

D: Cecum/Colon (150cm) pH 5.7~6.8

$\sim 10^{11}$ Bacteria/g

Bacteroides, Bifidobacterium, Eubacterium

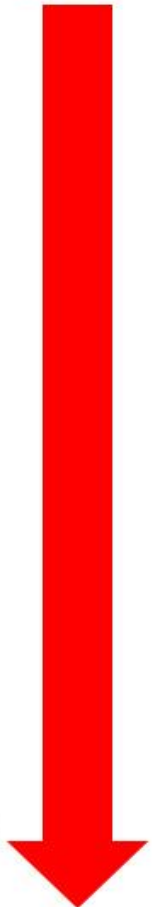
Ruminococcus, Peptostreptococcus

Propionibacterium, Clostridia, Lactobacillus

Escherichia, Streptococcus,

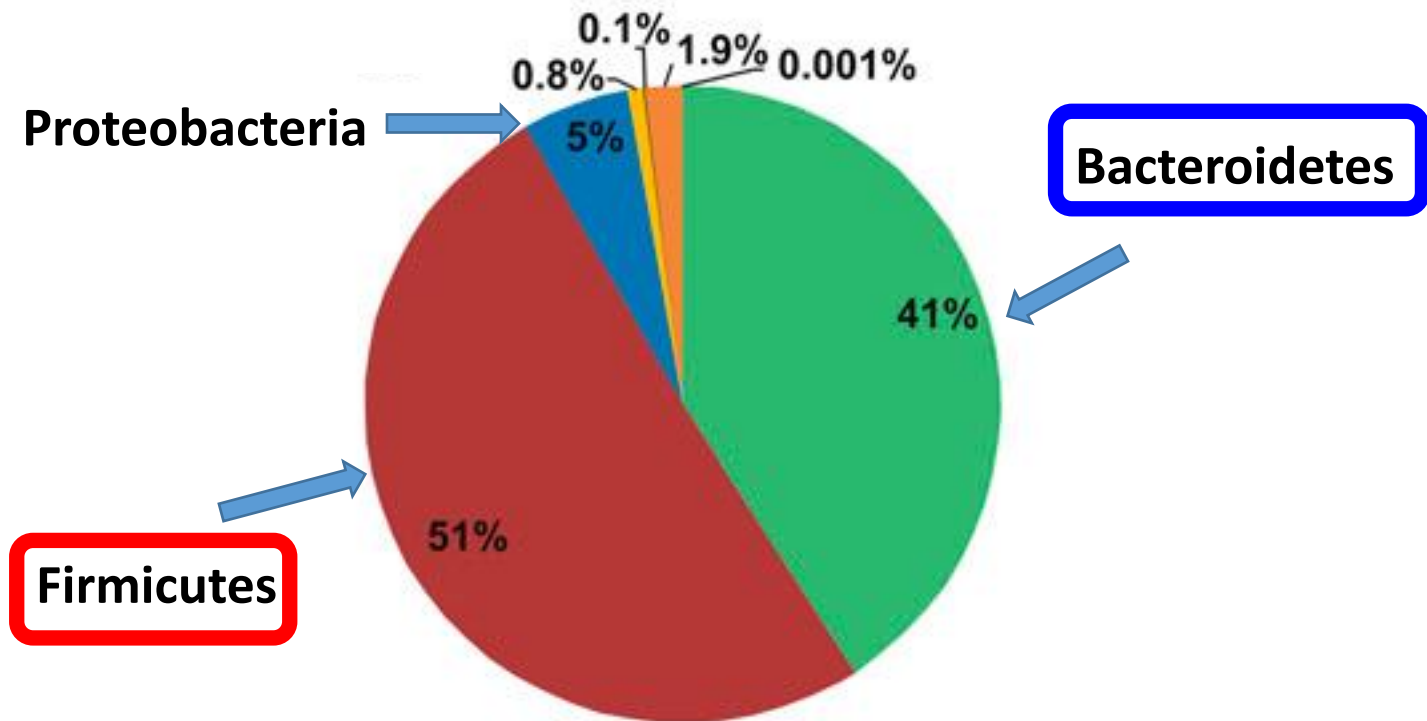
Methanobrevibacter

Low



High

Normal Gut Microbiota Composition at Phylum Level



Substrates for Intestinal Microflora

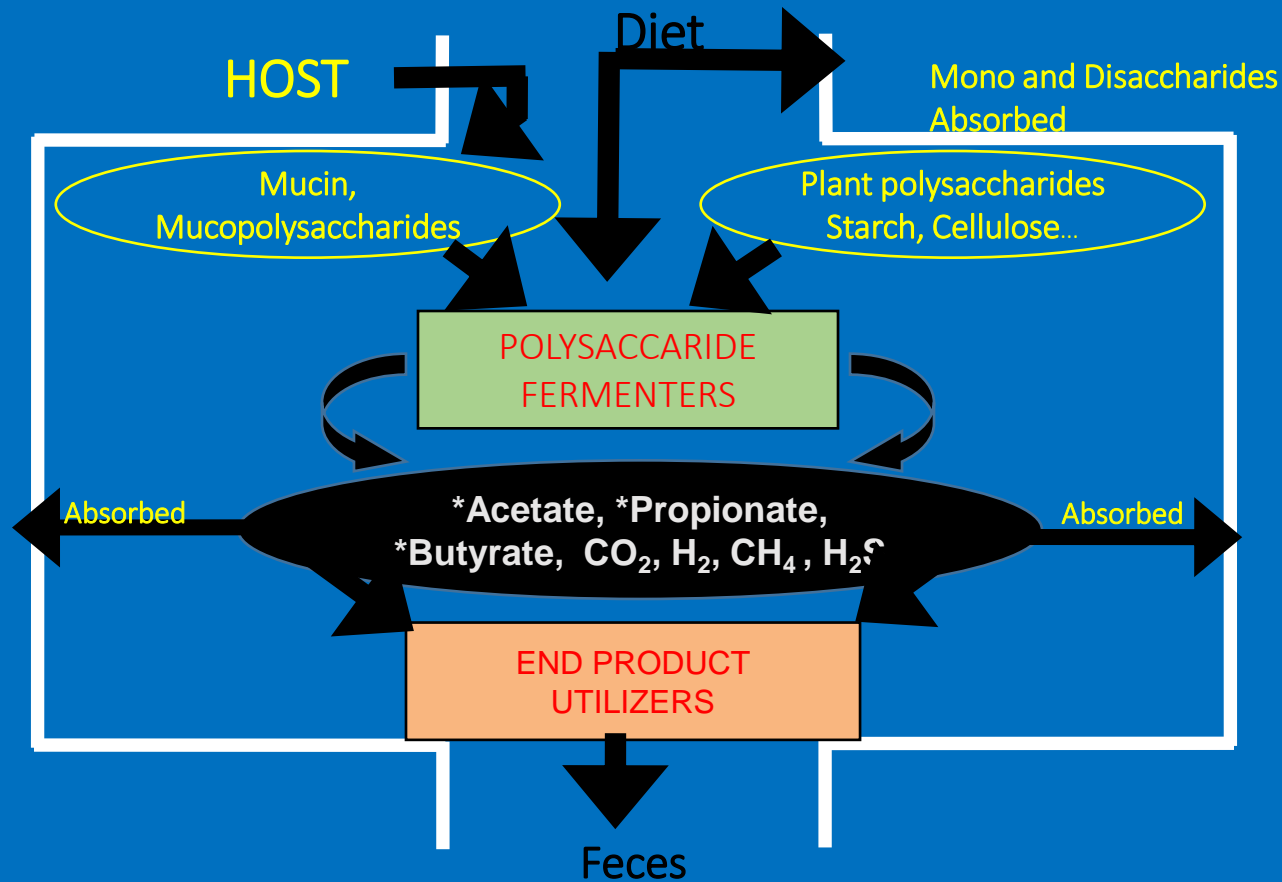
Endogenous:

**Sloughed intestinal cells (100-200g/d), bile
components**

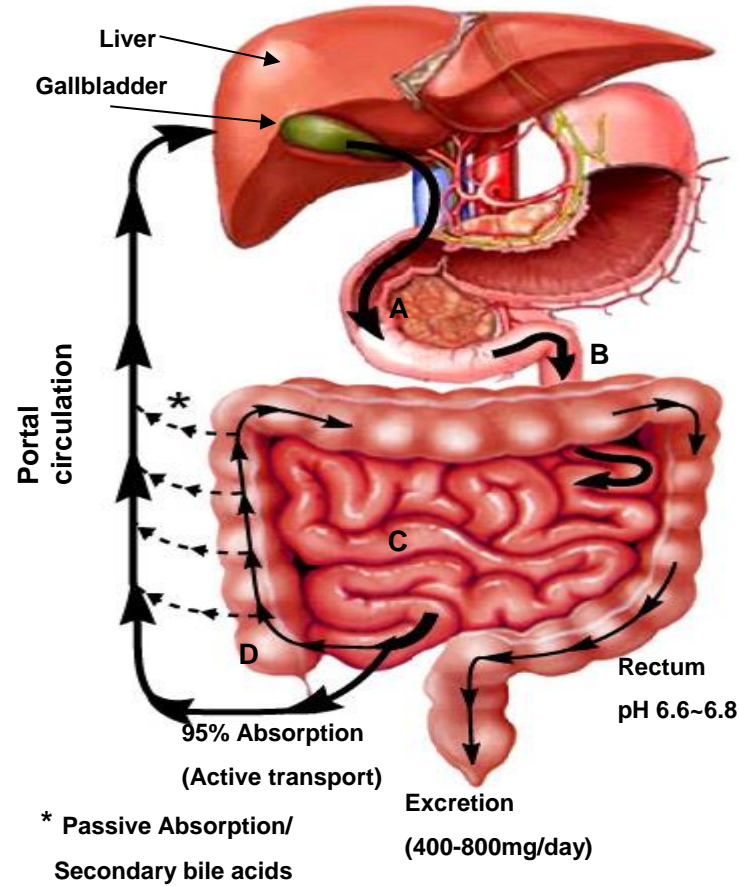
Exogenous:

**Resistant Starch; Plant polysaccharides; Proteins,
Sulfate**

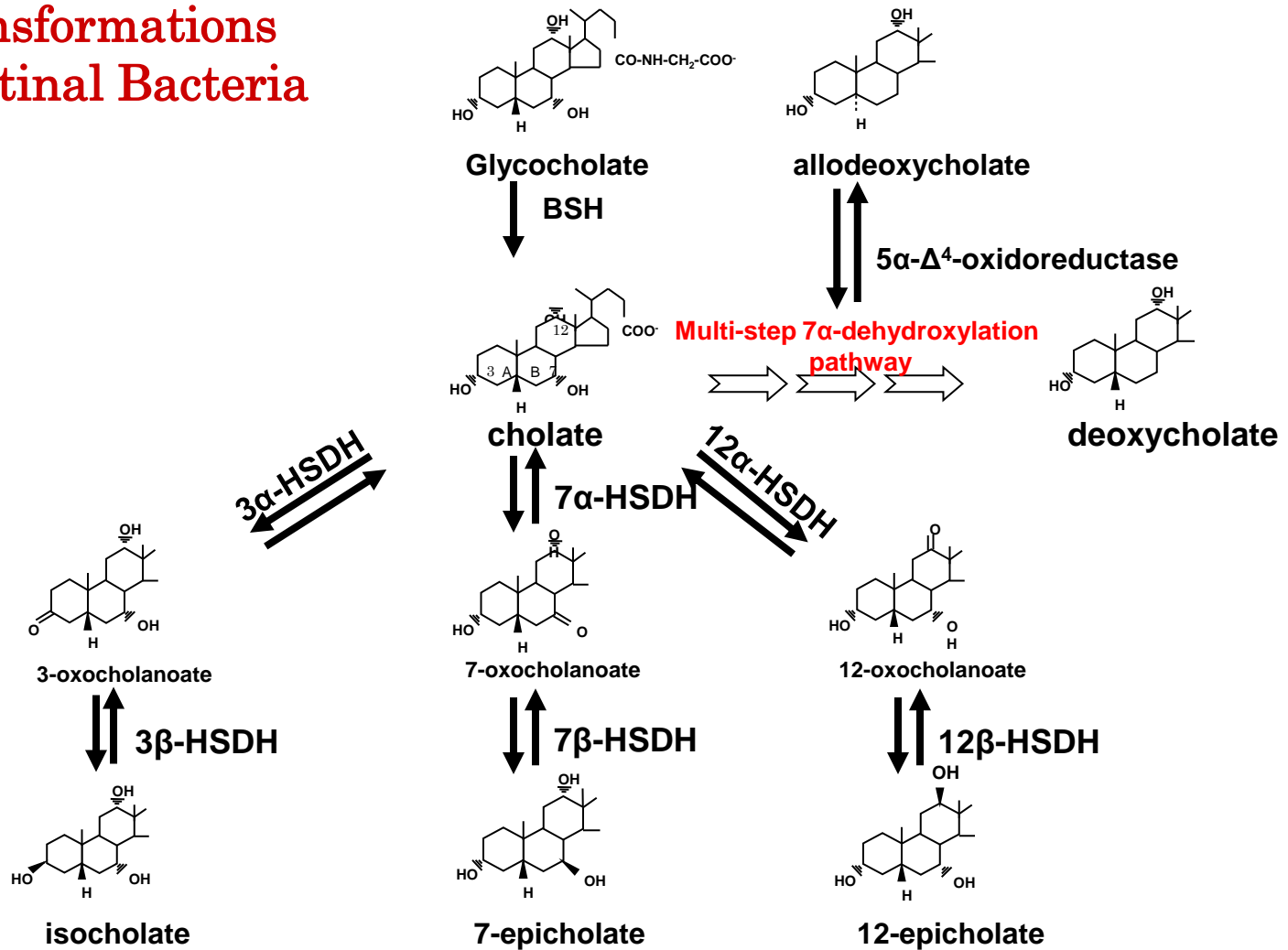
POLYSACCHARIDE FERMENTATION IN THE HUMAN COLON



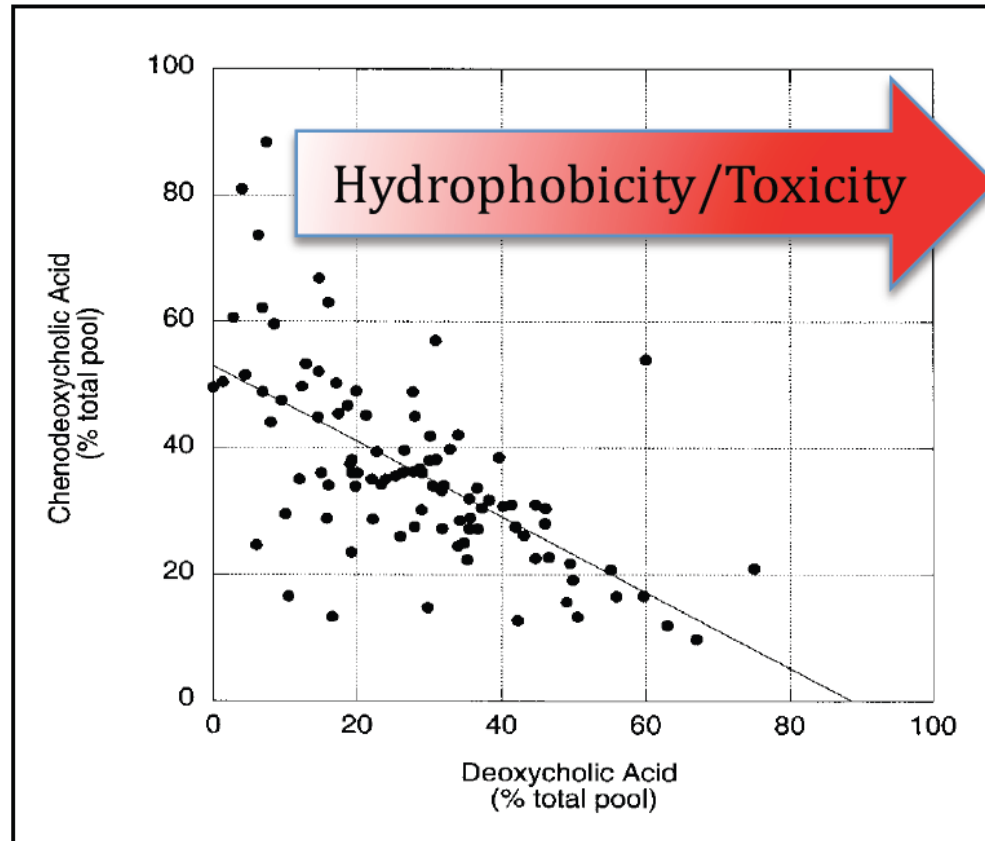
Enterohepatic Circulation Of Bile Acids



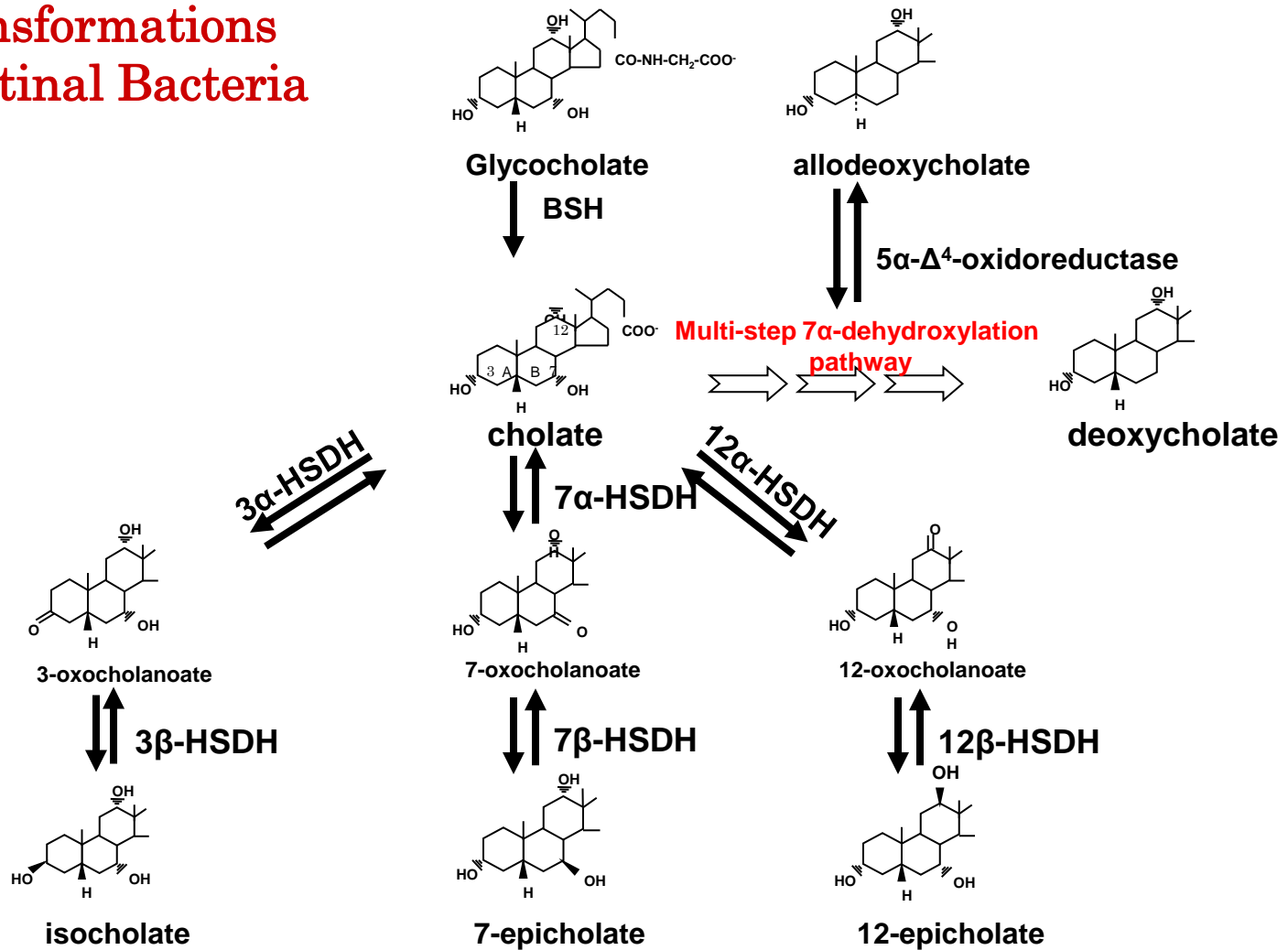
Bile Salt Biotransformations By Intestinal Bacteria



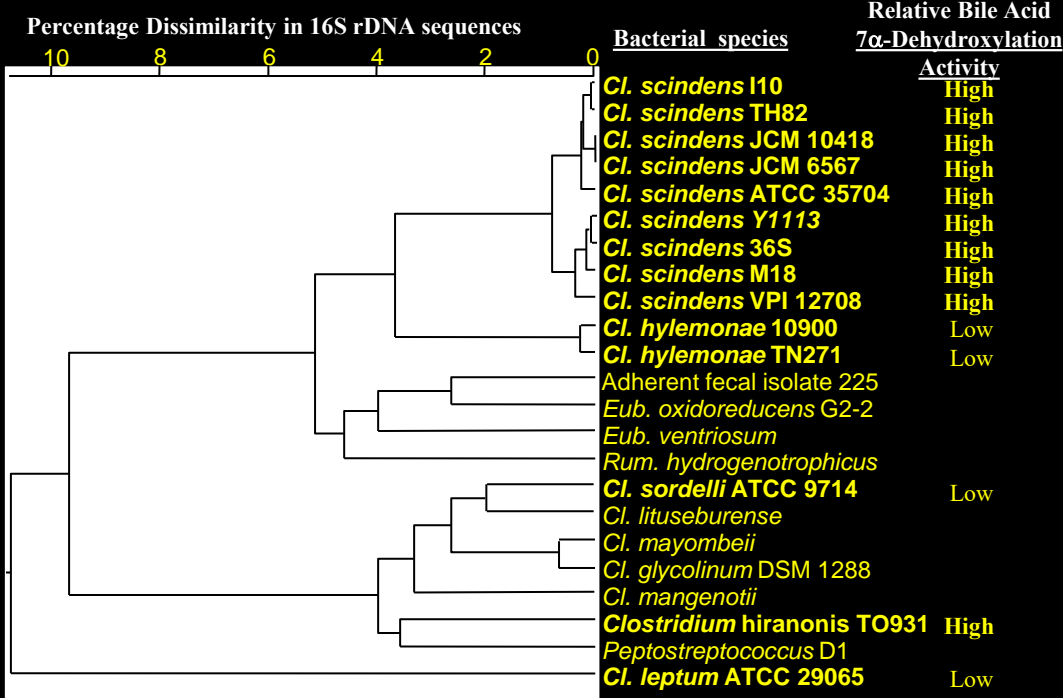
Accumulation of Deoxycholic Acid in Human Bile: Absence of Bile Acid 7 α -Hydroxylation in Human Liver



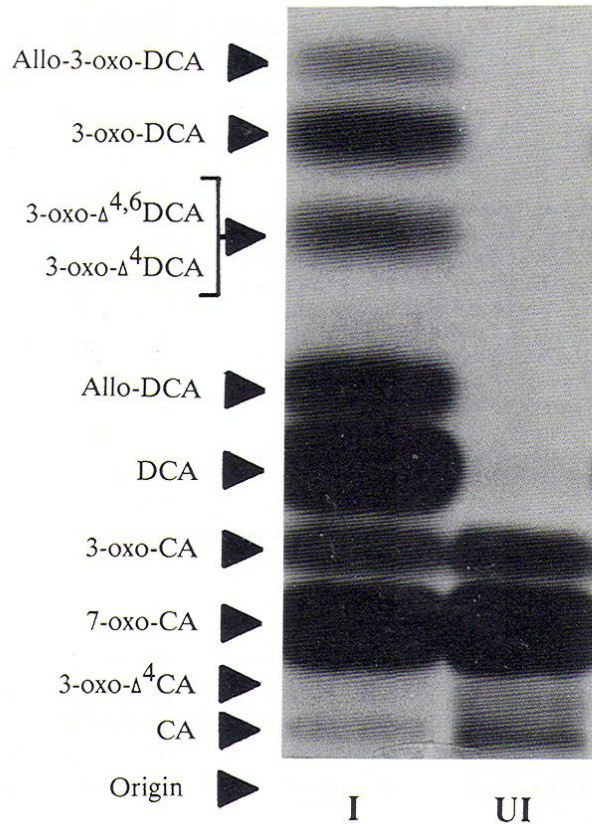
Bile Salt Biotransformations By Intestinal Bacteria



Phylogenetic analysis of cholic acid 7 α -dehydroxylating bacteria

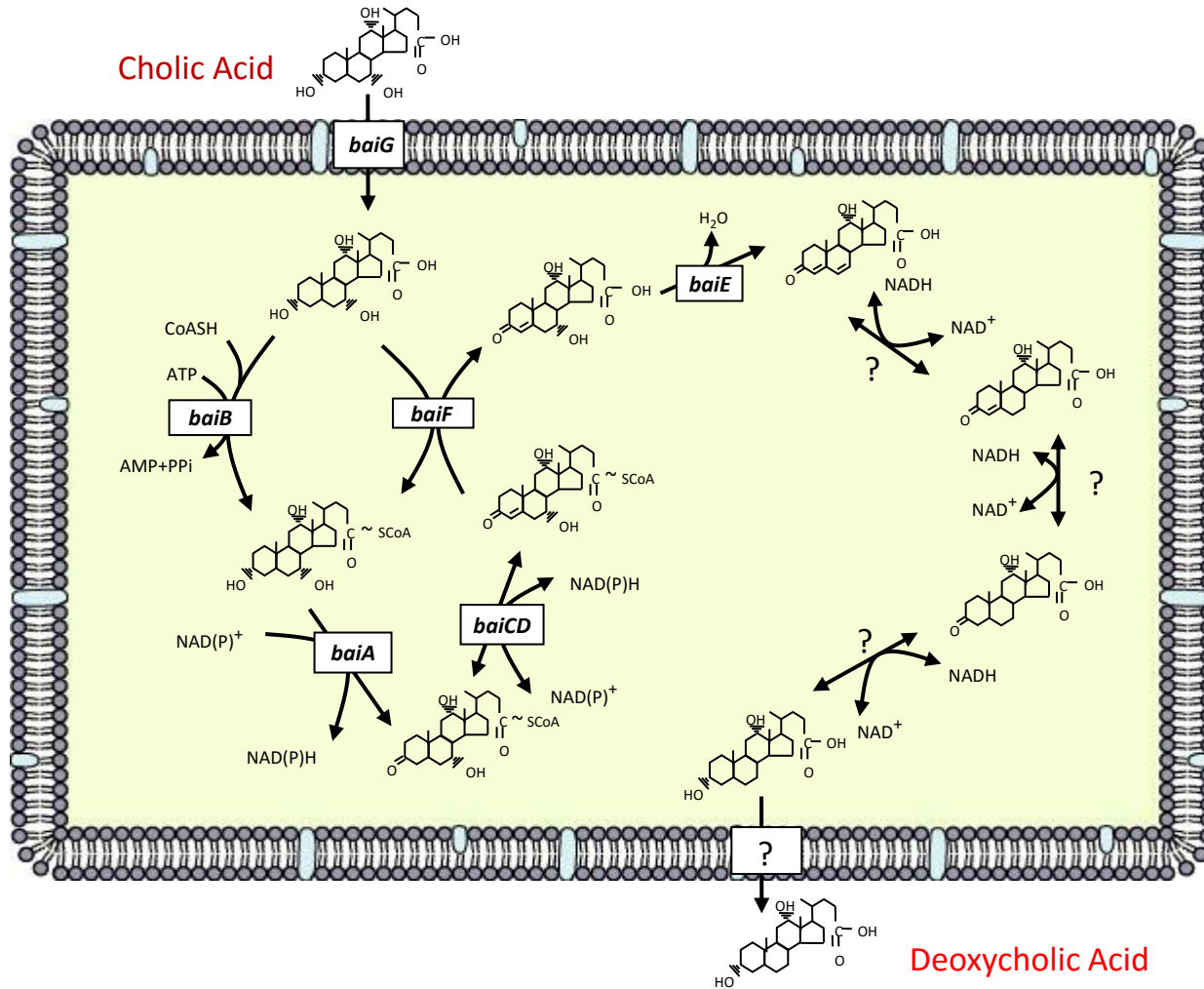


Intermediates in the bile acid 7 α -dehydroxylation pathway in *C. scindens*

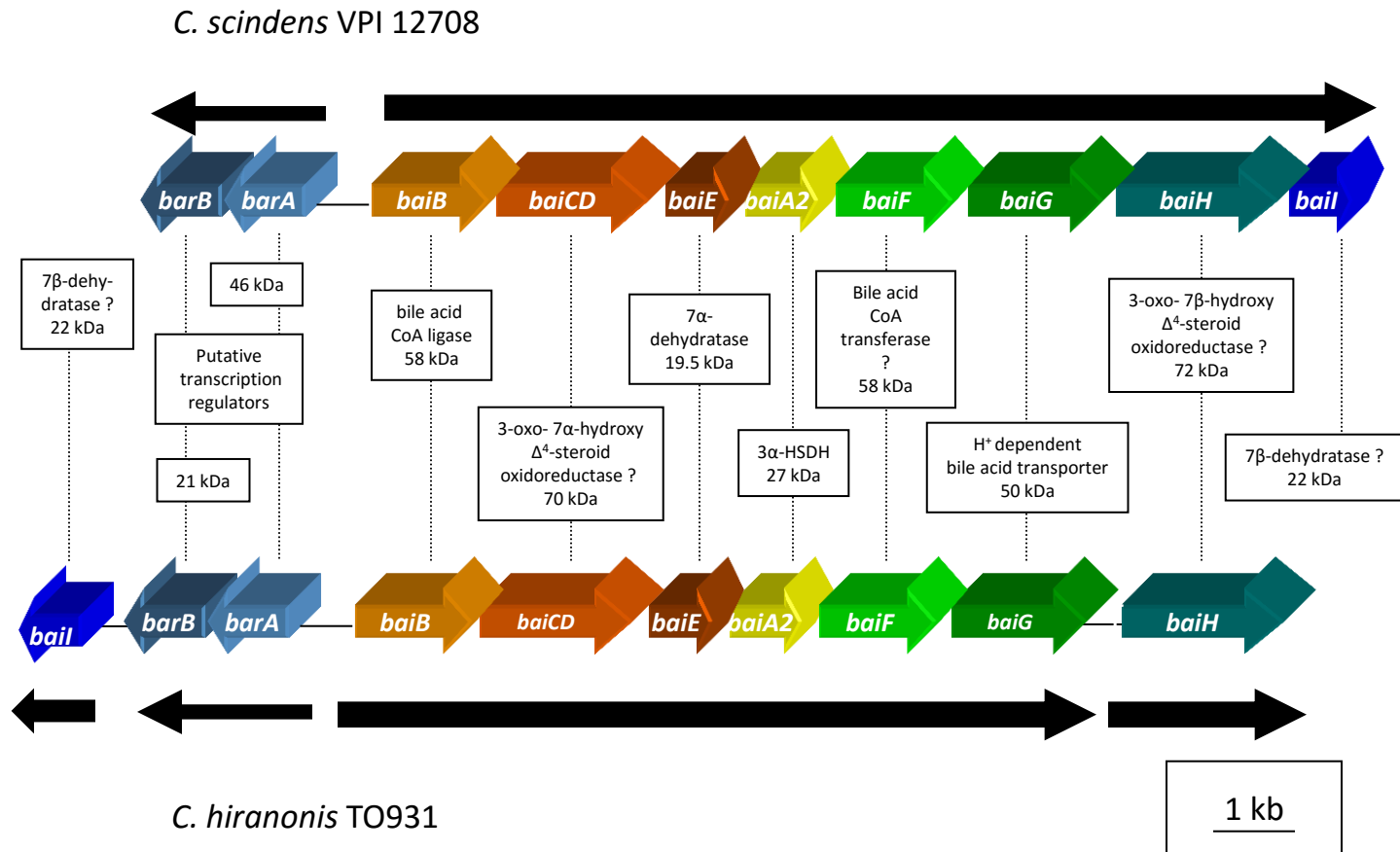


- ^{14}C CA incubated with Cell-extracts of *C. scindens* Prepared from CA induced Or uninduced control
- Products scraped from TLC and identity determined by stereospecific hydroxysteroid dehydrogenases, and mass spectrometry

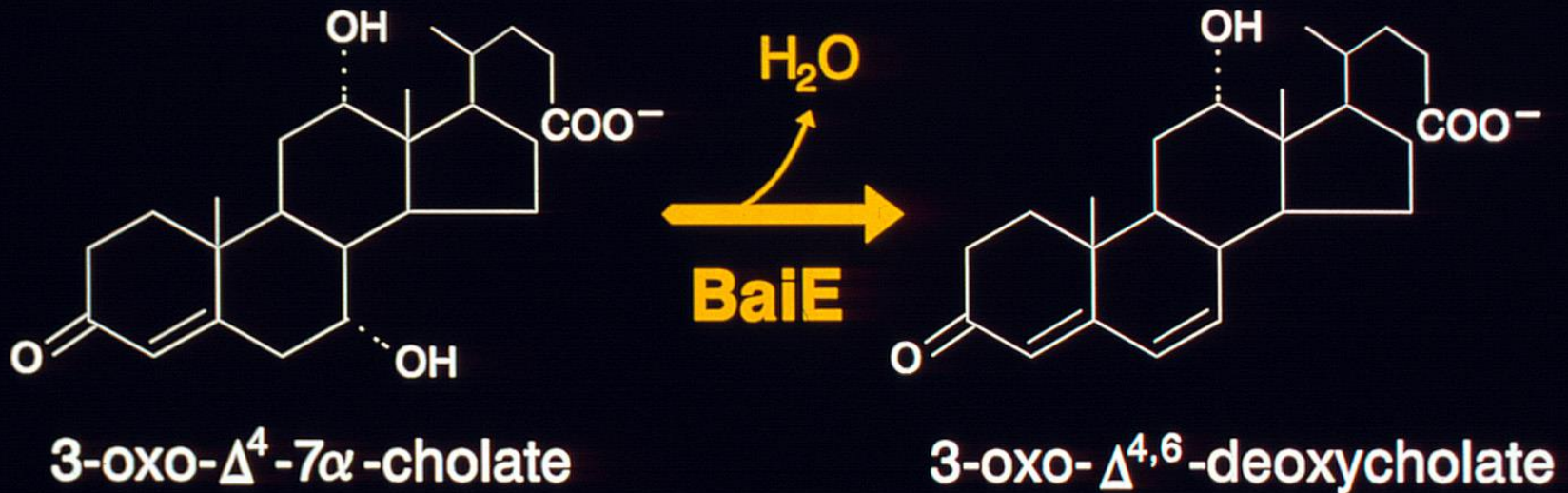
The Bile Acid 7 α -dehydroxylation Pathway in *Clostridium scindens*



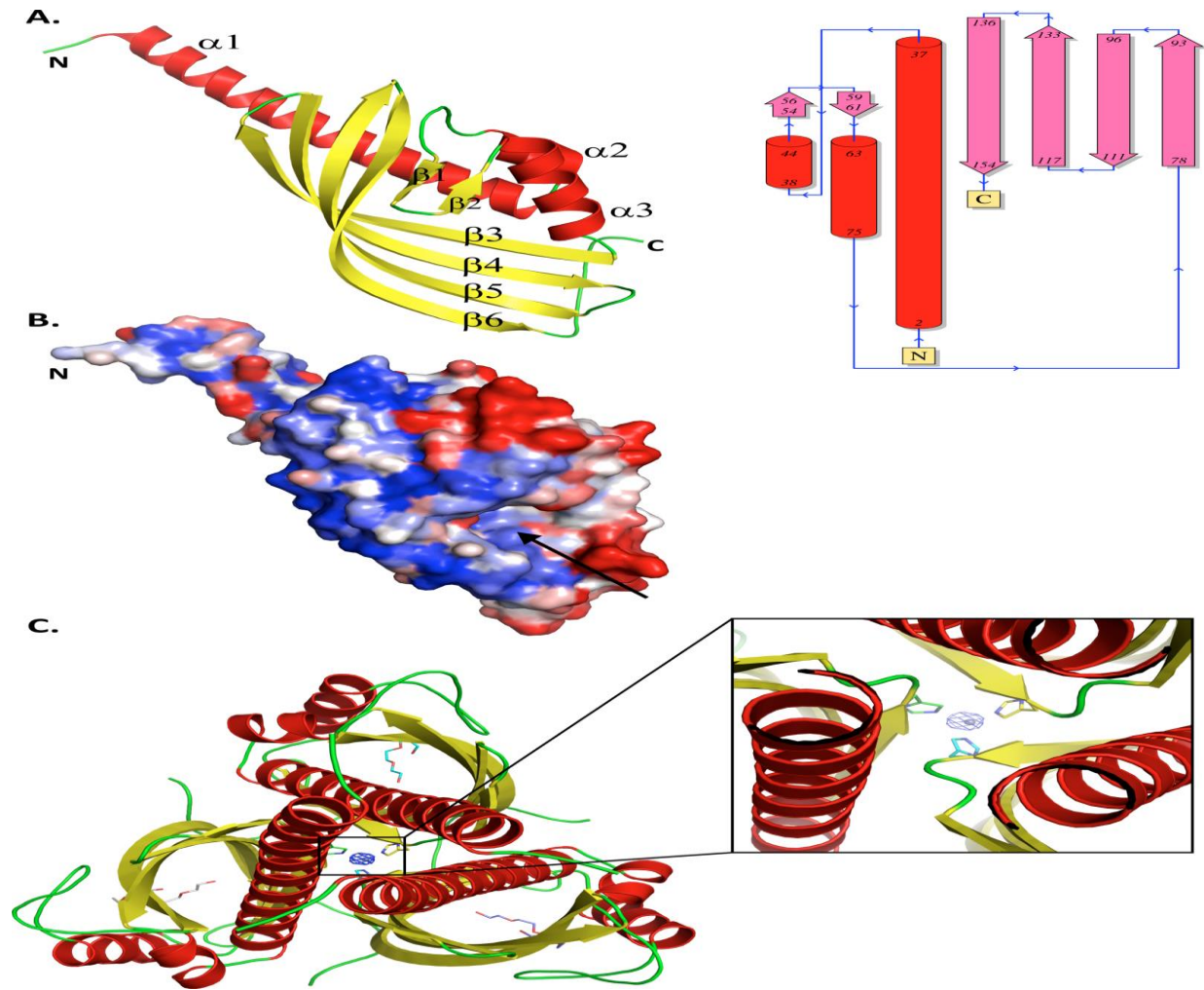
Bile Acid Inducible Operons Encoding Enzymes in the 7 α -dehydroxylation Pathway



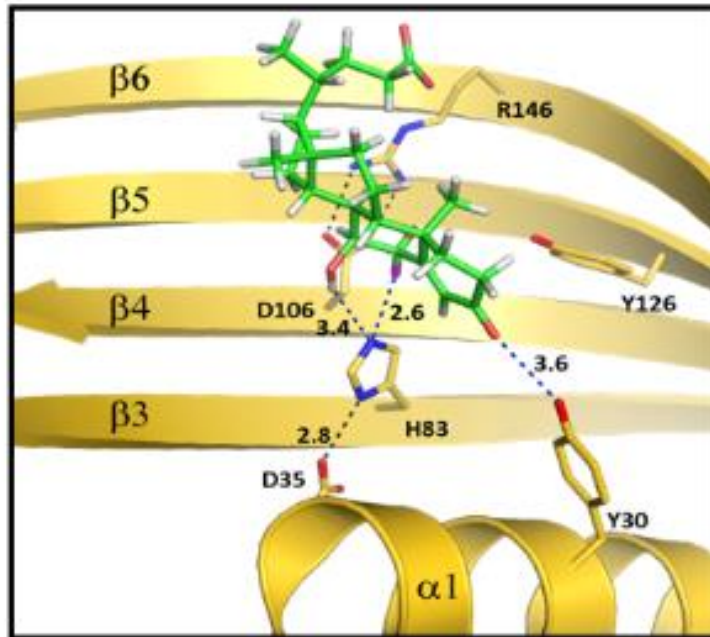
Bile Acid 7α -Dehydratase Reaction



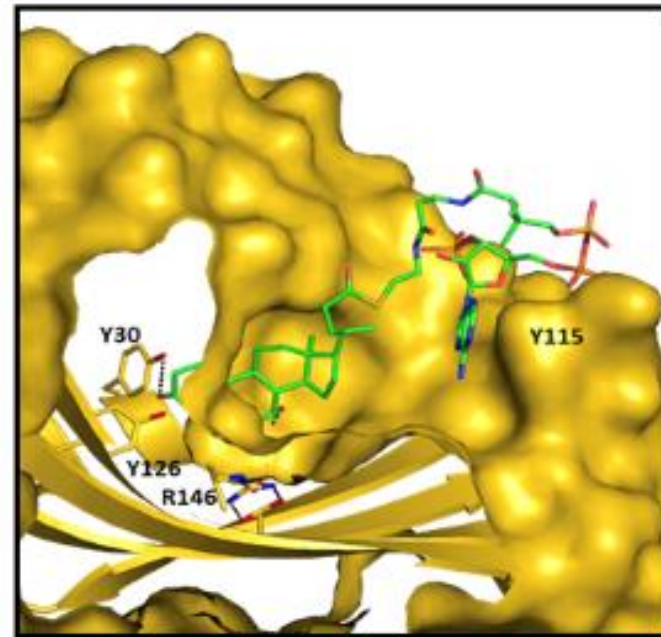
Structure of Bile Acid 7 α -Dehydratase



Active Site of Bile Acid 7- α -Dehydratase



A



B

What controls the levels of deoxycholic acid in human bile?

1. Numbers and activities of cholic acid 7 α -dehydroxylating bacteria in GI tract

➤ Berr F. et al. *Gastroenterology*. 1996; 111:1611-20

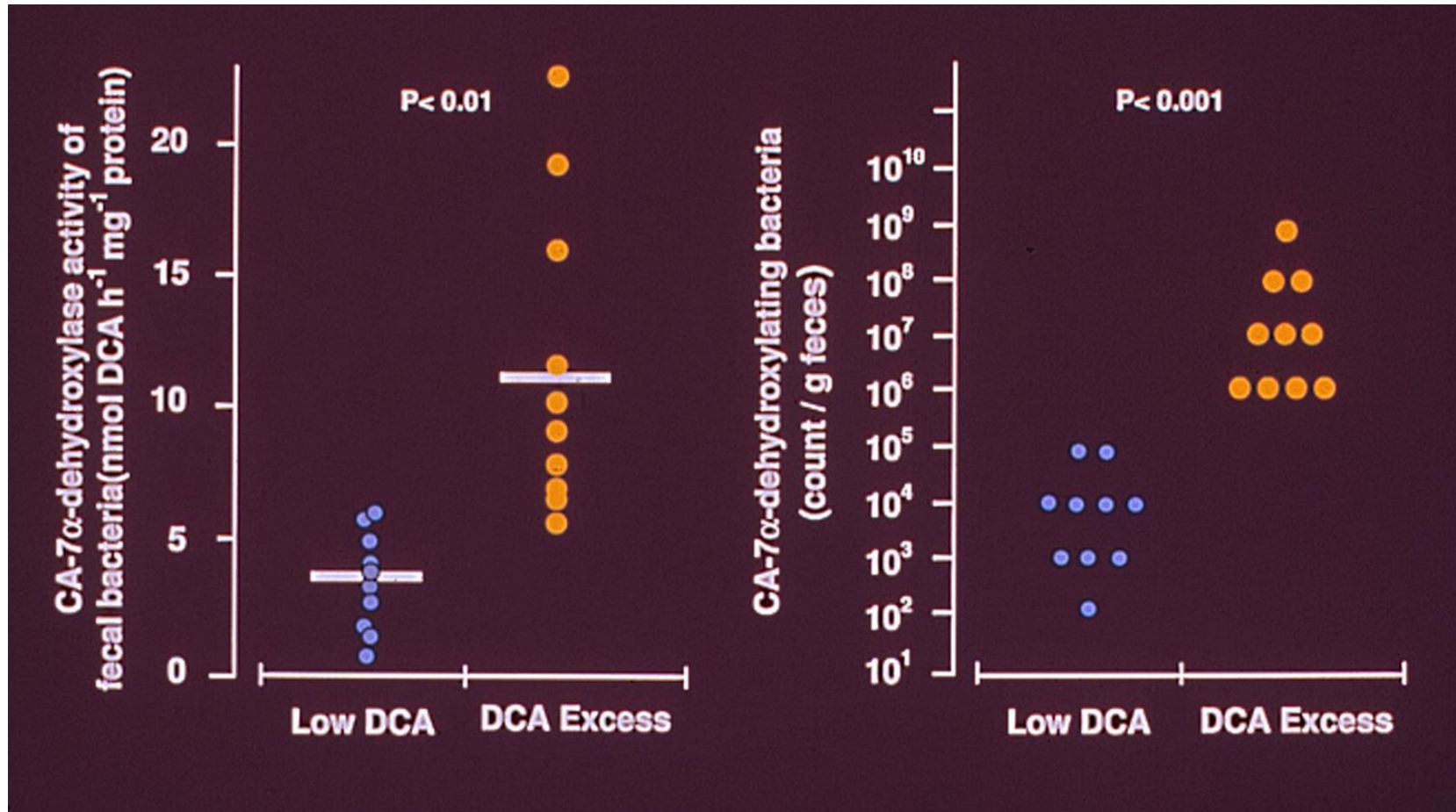
2. Intestinal transit time

➤ Thomas, L.A. et al. *Gastroenterology*. 2000; 119:806-15

3. Western diet

➤ O'Keefe S. J. et al. *Nat. Commun.* 2015; 6:6342

ACTIVITIES AND LEVELS OF CHOLIC ACID 7 α -DEHYDROXYLATING BACTERIA IN CHOLESTEROL GALLSTONE PATIENTS WITH DIFFERENT LEVELS OF DEOXYCHOLIC ACID



What controls the levels of deoxycholic acid in human bile?

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➤ Berr F. et al. *Gastroenterology*. 1996; 111:1611-20

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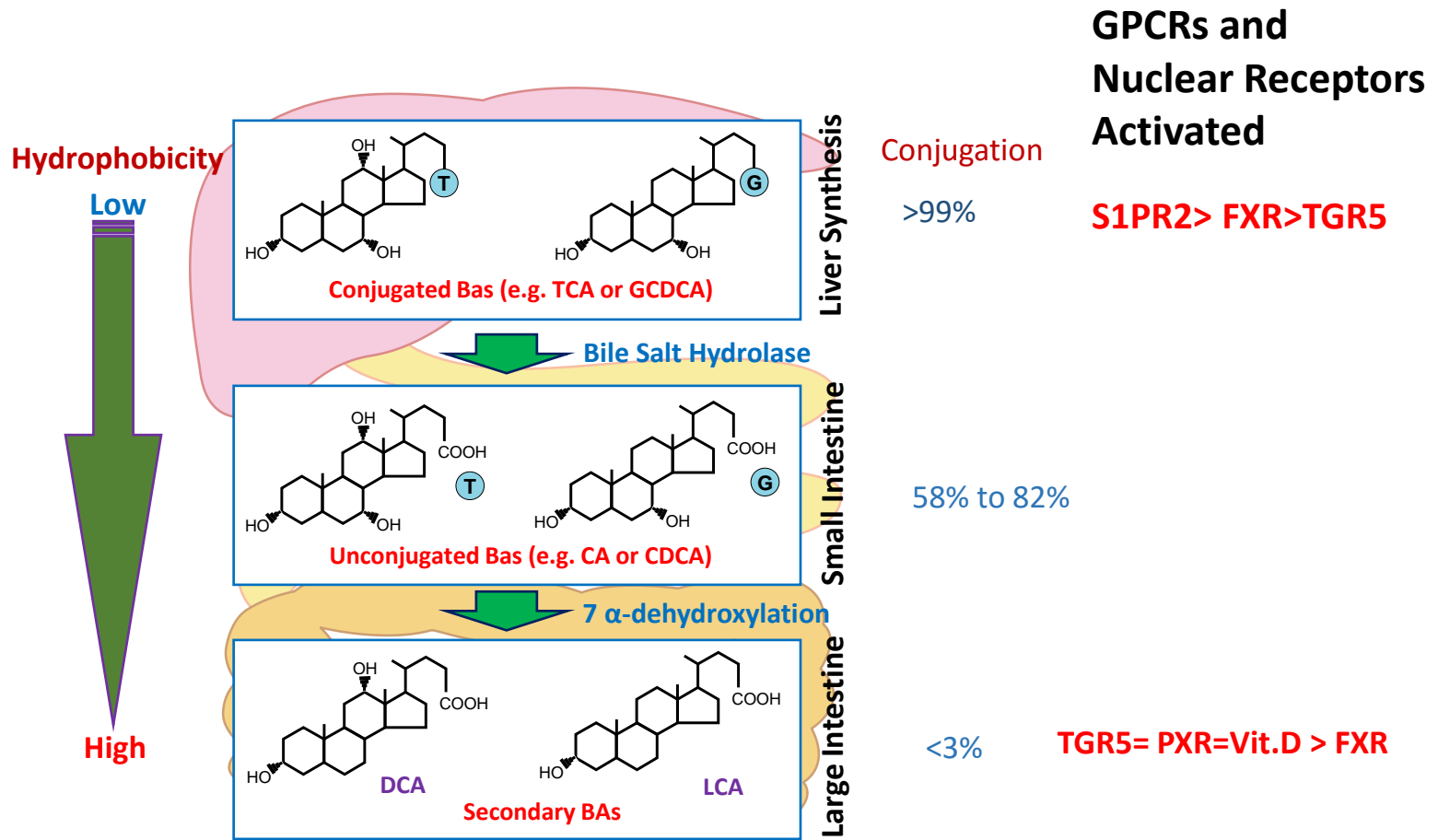
Bile Acids are Hormones and Gut Bacteria Control the Bile Acid Pool Composition

- Implications for Regulation of Hepatic Physiology and Metabolism

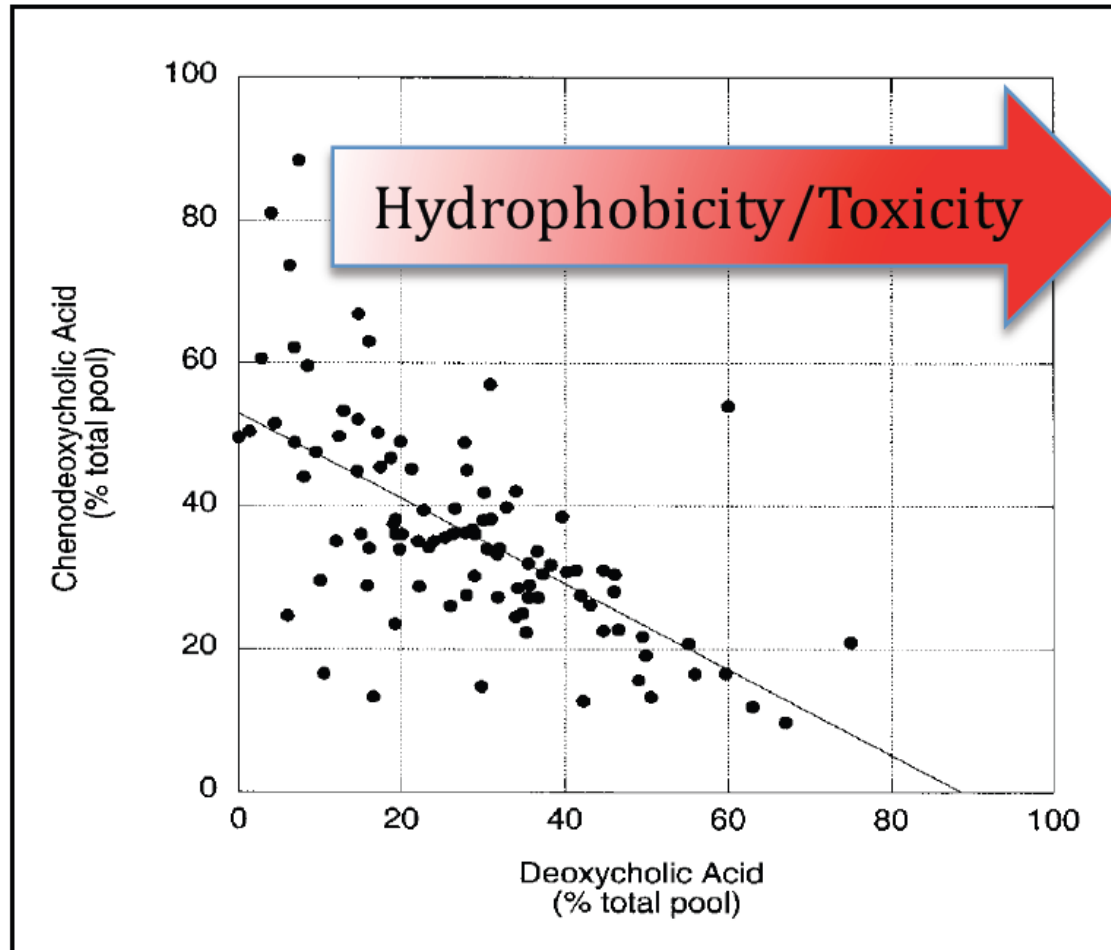
Effect of Bile Acid Structure on Activation of Nuclear Receptors and G-protein Coupled Receptors

Nuclear receptor	Bile acid agonist
Farnasoid X Receptor	CDCA > LCA = DCA > cholic acid
Pregnane-activated receptor	LCA > DCA > cholic acid
Vitamin D receptor	3-oxo-LCA > LCA > DCA > cholic acid
G-protein coupled receptors	
TGR-5	DCA > LCA > CDCA > cholic acid
Sphingosine-1-phosphate receptor 2	Taurine or glycine conjugated
(M _{2,3}) muscarinic receptors	T-LCA > T-DCA > T-cholic acid

Microbial Bile Acid Gateway Reactions



Accumulation of Deoxycholic Acid in Human Bile is Associated with Increased Risk of **Colon Cancer** and Possibly **Liver Cancer**:
Absence of Bile Acid 7 α -Hydroxylation in Human Liver



Association of Deoxycholic Acid and Colon Cancer

1. Found in fecal water 100-300 μM in high risk individuals
2. Can cross colonocyte plasma membranes without transporter
3. Activates cell signaling pathways associated with tumorigenesis e.g. Activates the Epidermal Growth Factor Receptor (EGFR) \rightarrow AKT, ERK1/2 \rightarrow induces COX-2; β -catenin, Protein Kinase C
4. Increased in “Western type” diets.
5. Found in significantly higher concentrations in blood, bile and feces of colon cancer patients and patients with polyps vs controls.
6. Animal model studies show promotion of colon cancer by deoxycholic acid.

Deoxycholic Acid Activates the EGFR

Possible Mechanisms of Activation

1. Stimulates superoxide radicles that inhibit phosphotyrosine phosphatases

➤ Qiao L. et al. Mol Biol Cell 2001; 12(9);2629-45

2. Activates matrix metalloproteinases releasing EGFR ligands i.e. TGF α , amphiregulin

➤ Werneburg N.W. et al. A. J. Physiol Gastrointest Liver Physiol 2003; 285:G31-36

3. Increases intracellular calcium preventing EGFR degradation.

➤ Centuori S. M. et al. BBA Molecular and Cell Biology; 2016; S1388-1981:30100-7

Deoxycholic Acid and Liver Cancer

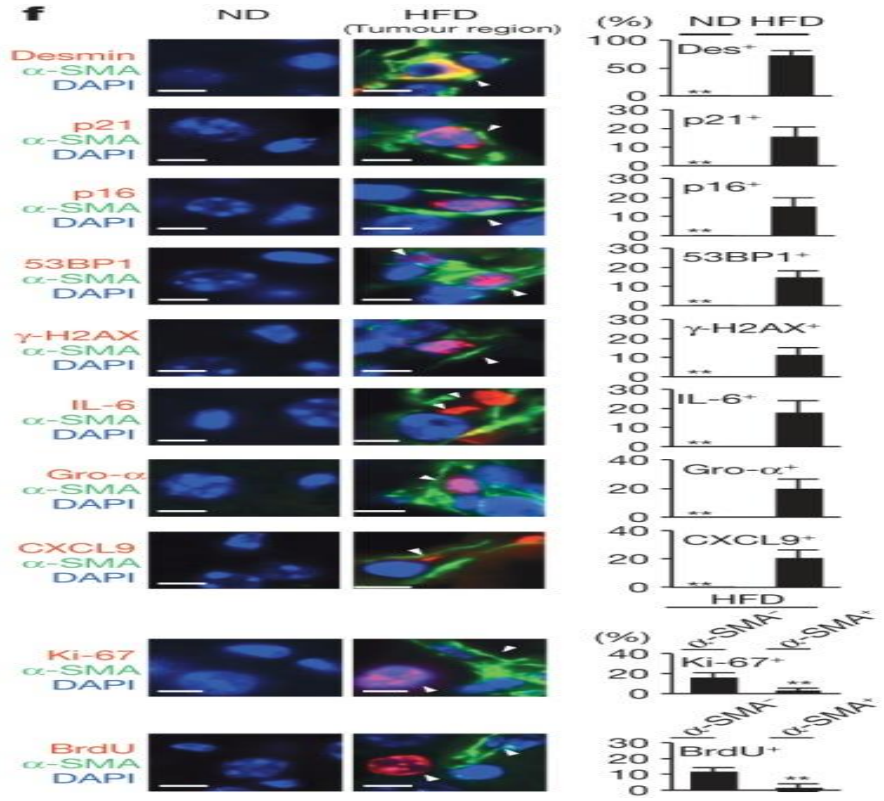
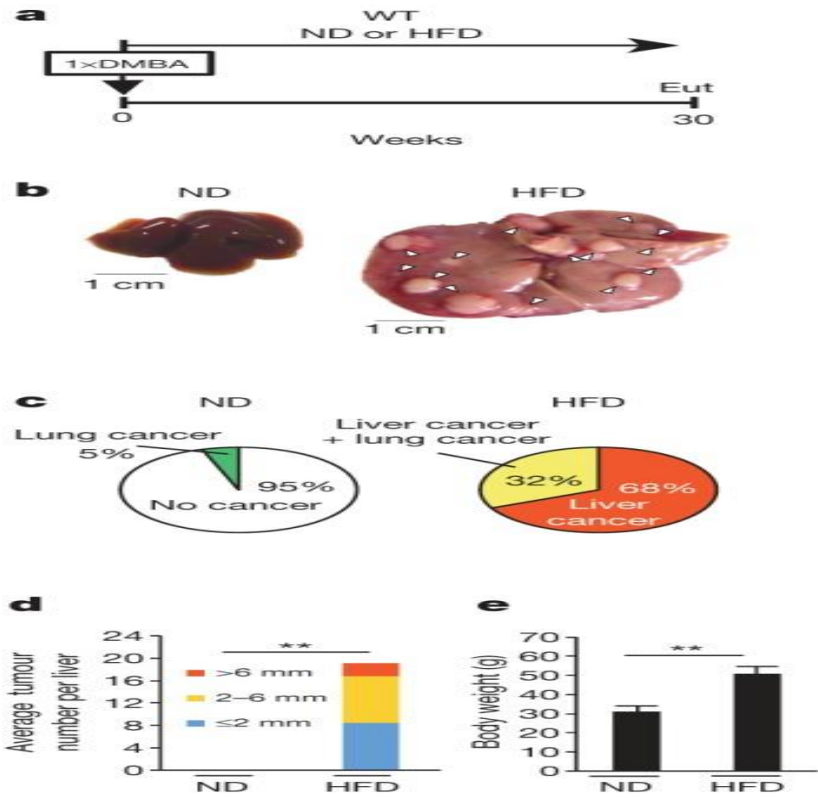
Yoshimoto S. et al. 2013, Nature; 499(7456)97-101

Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome

Yoshimoto S. et al. 2013, Nature; 499 (7456) 97-101

“Senescent cells often develop a secretory profile composed mainly of inflammatory cytokines, chemokines and proteases, a typical signature termed senescence-associated secretory phenotype (**SASP**)”.

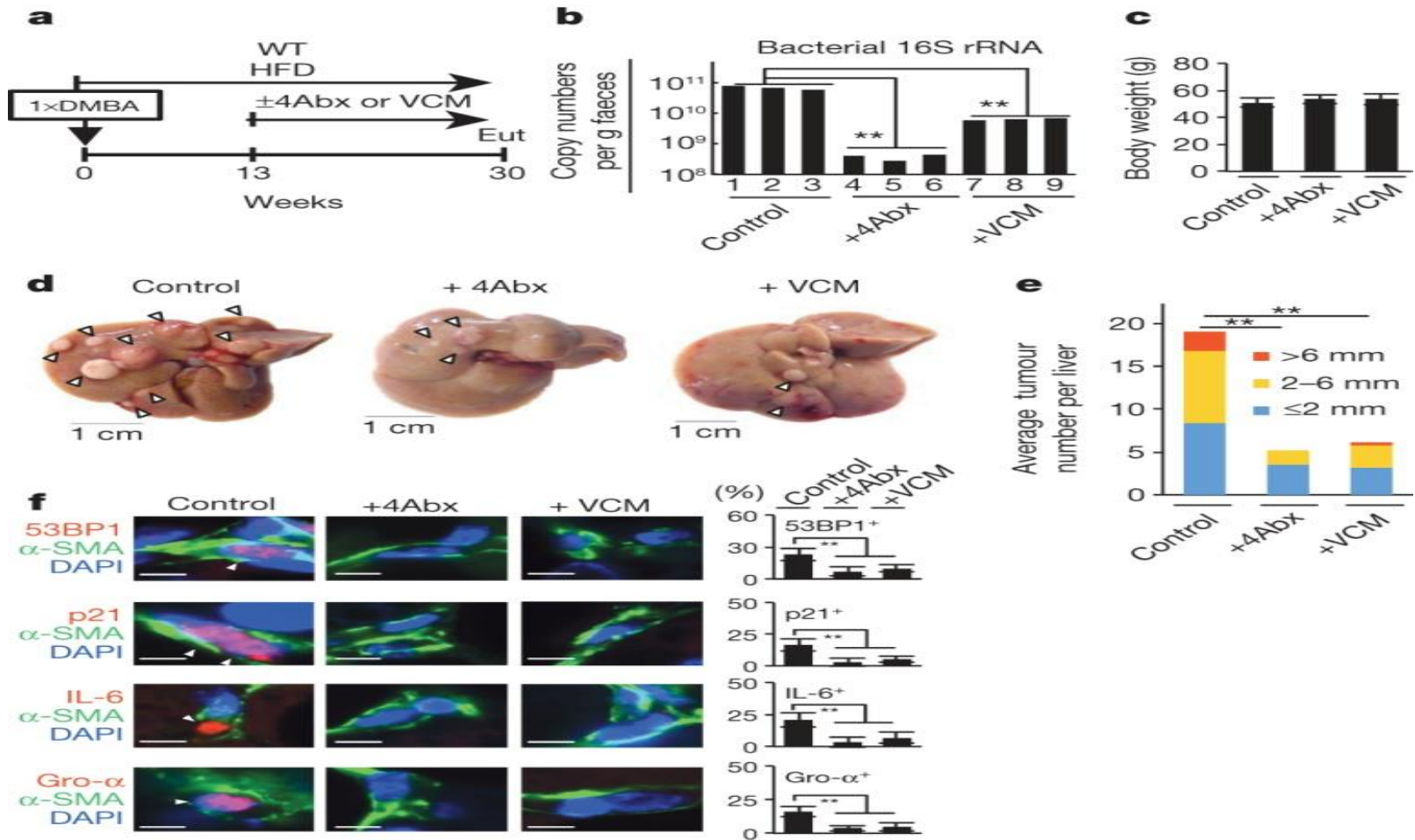
Cellular Senescence in Hepatic Stellate Cells



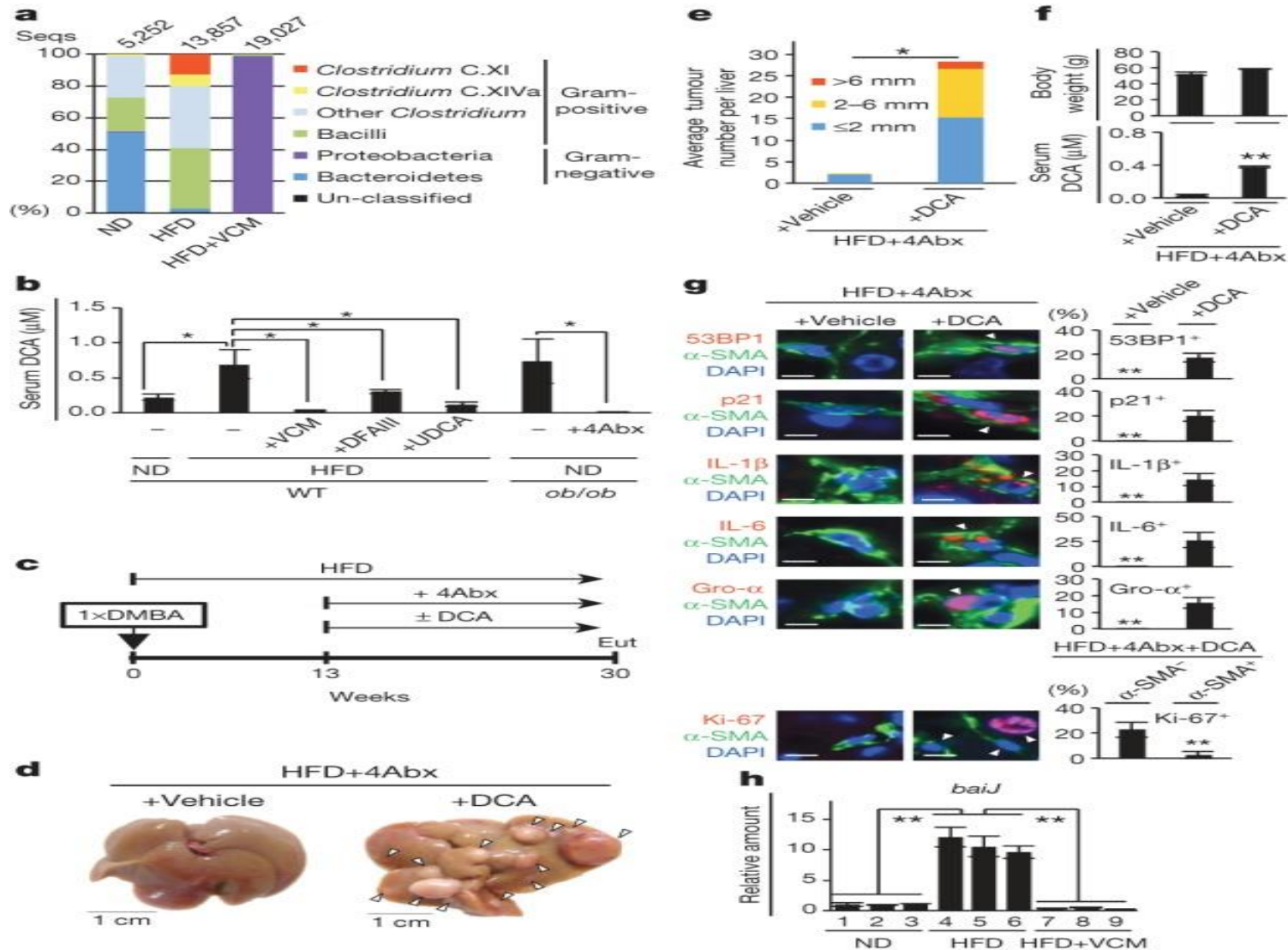
nature

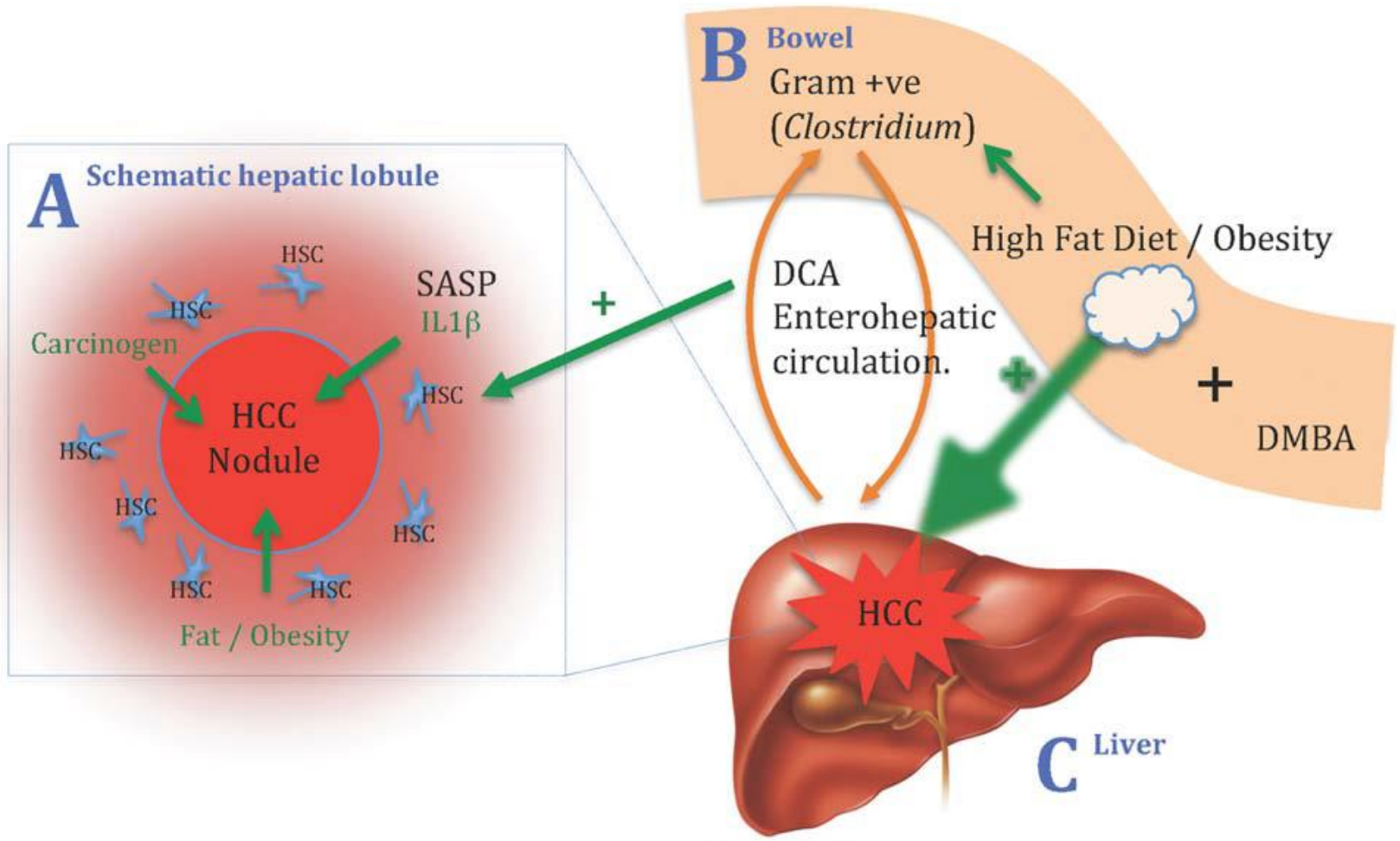
Yoshimoto S. et al. (2013) Nature

Antibiotic Treatments Alleviate Obesity-induced HCC Development



Bacterial metabolite promotes obesity-induced HCC development





Summary

- 1. The human bile acid pool composition is regulated by diet, intestinal transit time and gut microbiota. “Western diets” shifts bile acid conjugation to taurine and increases deoxycholic acid levels in humans.**
- 2. The bile acid pool composition and conjugation alters the activation of specific nuclear receptors (FXR, Vitamin D, PXR) and GPCRs (TGR5, S1PR2) regulating hepatic metabolism and inflammation.**
- 3. Increased levels of deoxycholic acid in the human bile acid pool is associated with increased hydrophobicity, inflammation, and cancers of the liver and colon**

Our Research Team



Our Research Team

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- Scripps Research Institute
 - Shiva Bhowmik, Ph.D.
 - Scott Lesley, Ph.D.
-
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