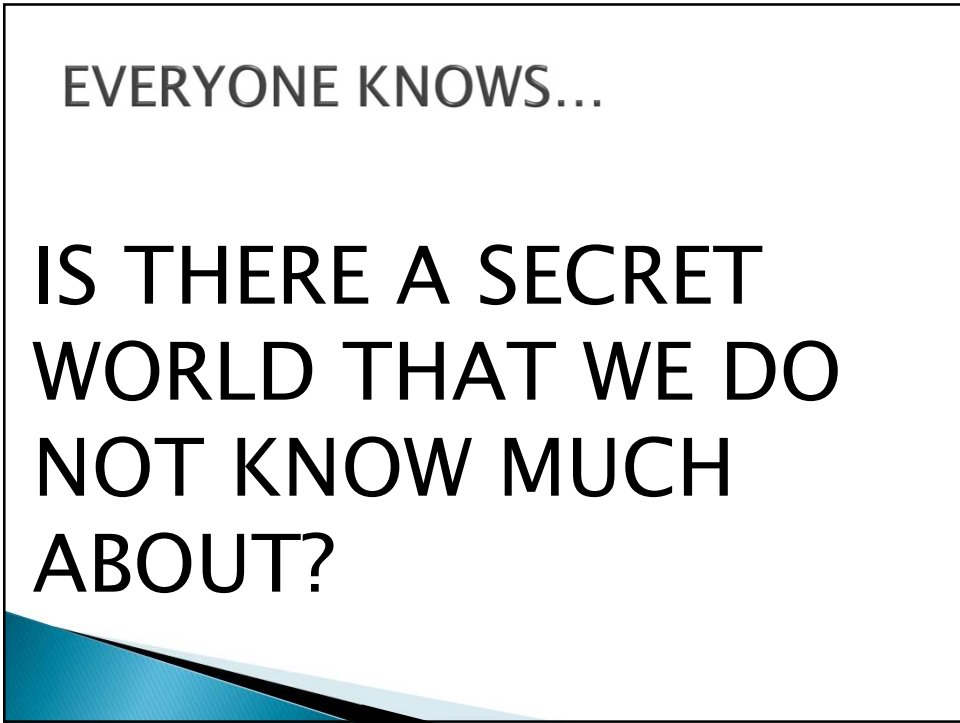


**GASTROINTESTINAL
PROCESSES**

**Beyond Probiotics:
The Secret World of Small Molecules**

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EVERYONE KNOWS...

**IS THERE A SECRET
WORLD THAT WE DO
NOT KNOW MUCH
ABOUT?**

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20th CENTURY MEDICINE

- ▶ Up to 1953, biological research focused on organismal perspective.
- ▶ 1953 – landmark discovery of the structure of DNA. Research shifted from an organismal to a molecular perspective - the role played by macromolecules— not only DNA, but also RNA and proteins
- ▶ Discovering genomes was believed to have capacity to usher a new era of eradicating most diseases

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HUMAN GENOME PROJECT 20/21

- ▶ 1990–2003 International effort to sequence the 3 billion DNA letters in the human genome
- ▶ HGP was not able to sequence all of the DNA found in human cells; the aim was to sequence only euchromatic regions of the nuclear genome, which make up 92.1% of the human genome. The remaining 7.9% exists in scattered heterochromatic regions such as those found in centromeres and telomeres.
- ▶ With the technology that could handle repetitive sequences, scientists finally filled those gaps in May 2021, and the first end-to-end human genome was [officially published on Mar. 31, 2022](#).
- ▶ Knowledge of all the human genes created new opportunities for developing novel **DRUGS**
- ▶ Discovering genomes was believed to have capacity to usher a new era of eradicating most diseases

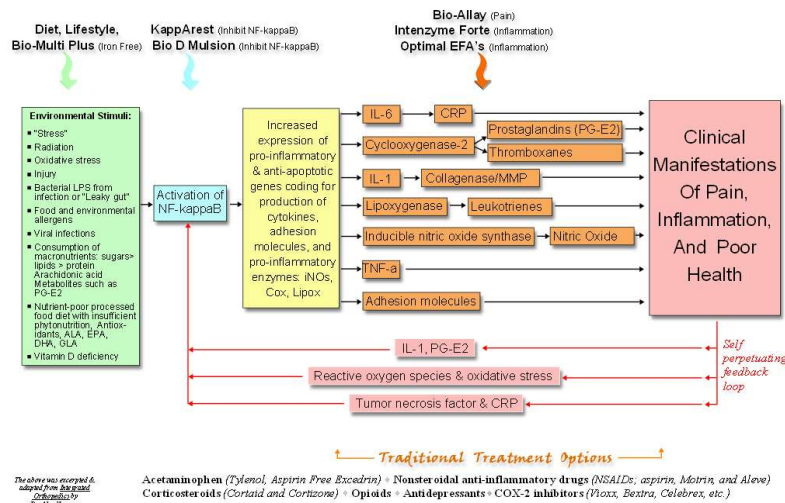
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21ST CENTURY MEDICINE

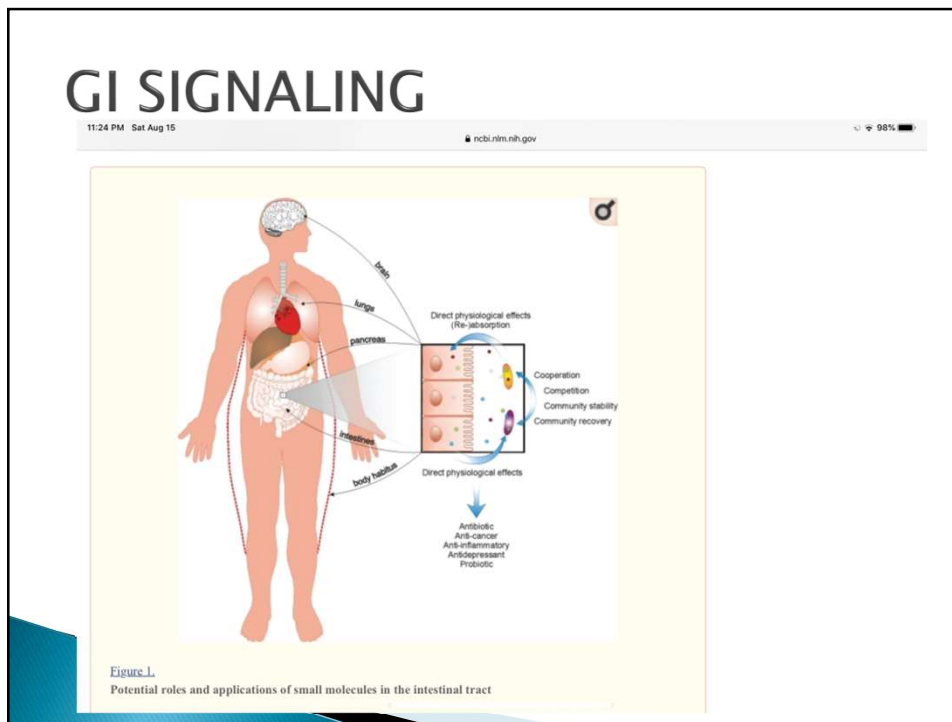
- ▶ Small molecules importance and interactions
- ▶ Importance of finding ways to express or shut off GENE EXPRESSION
- ▶ NF KAPPA B – transcription factor, a prime example

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The BioChemical Process Of Inflammation & Pain



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LIFE

▶ Three domain system

1. Eukaryota
2. Bacteria
3. Archaea

EUKARYOTS – cells have a nucleus within a nuclear envelope

PROKARYOTS – lack cell nuclei

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SIGNALING in the GI tract

- ▶ Our knowledge on cellular chemical communication comes from studies of multicellular eukaryotes, but bacteria can also communicate using sophisticated signaling systems
- ▶ Every living cell produces hormone-like diffusible small molecules that can be used to convey messages to neighboring cells—a vital step in adaptation, development, and survival within populations.
- ▶ There is a wealth of **UNIDENTIFIED bioactive small molecules** in the human body, originating from both microbial and human cells and that have important biological functions.

<https://pubmed.ncbi.nlm.nih.gov/21399765/>
F1000 BIOLOGY REPORTS

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- ▶ “Small molecules” - any compound of molecular weight around or under 3000 Da and with chemical characteristics that preclude their description as DNA, RNA, or proteins.
- ▶ Critical biological functions in humans:
 - control immune functions,
 - the development of sexual characteristics,
 - stress responses,
 - metabolism,
 - mineral balance, amongst others
- ▶ In higher organisms, these small molecules are called hormones—from the Greek for “excite” or “arouse”—a term coined in 1905 by Ernest Starling
 - ▶ They are produced by one organ of the body and travel to distant organs to exert physiological effects.
 - ▶ Hormones and hormone-like molecules

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Small-molecule mediated microbe-host and microbe-microbe interactions

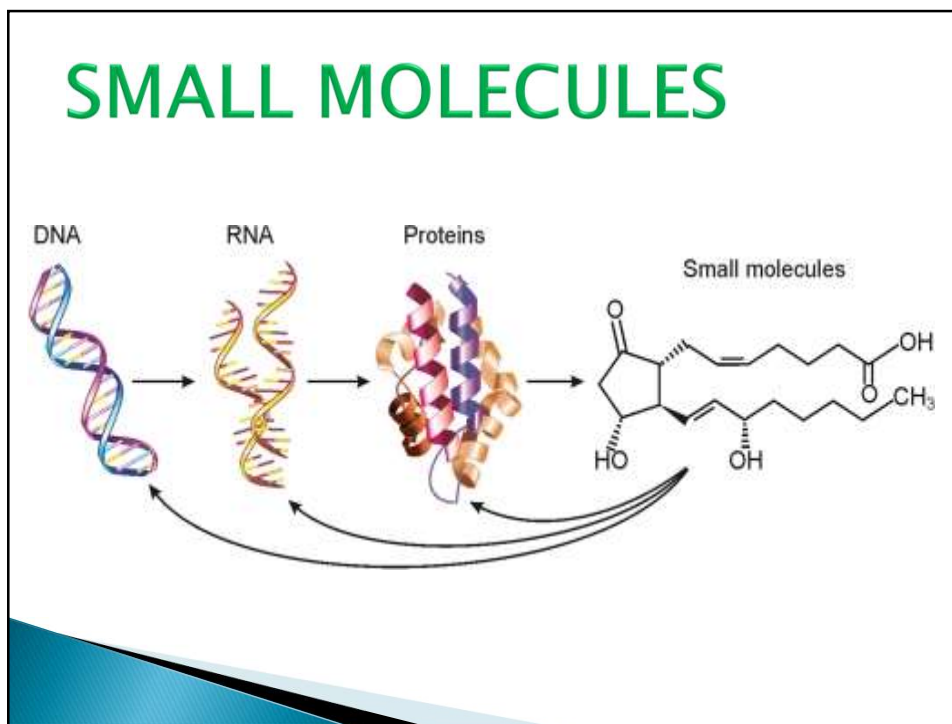
- ▶ The microbiota produce a range of small molecules from various classes with distinct targets.
- ▶ Four examples
 1. the nonribosomal peptide **tilivalline**, whose host target is unknown;
 2. the ribosomally synthesized and post-translationally modified peptide **microcin E492** (MccE492), a narrow spectrum antibacterial;
 3. **lipid A**, the glycolipid core of lipopolysaccharide, which targets TLR4 in host immune cells; and
 4. **indole propionic acid**, a reductive metabolite of tryptophan that enters host circulation but whose biological activity is poorly understood. These metabolites are each produced by different species of the microbiota

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It's a small-molecule world

- ▶ “Since its discovery, DNA has been considered the foundation of life. Its capacity to store information coupled with its remarkable stability make it the prime candidate for the molecule from which life, as we know it, originated. This concept is imprinted in the “central dogma”, which states that DNA holds all genetic information, which is passed on to RNA as a messenger molecule and then translated into proteins, which constitute the machinery and structures that carry out the molecular processes essential for life ([Figure 2](#)). Although generally accepted, this viewpoint has been challenged. In 1986, Walter Gilbert suggested that RNA preceded DNA as a self-replicating primitive form of life, giving this molecule a main role in the formation of life [[64](#)]. Indeed, RNA molecules with enzymatic functions still exist [[65](#)].”

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SMALL MOLECULES

- ▶ [Figure 2.](#)
- ▶ **Small molecules as important messengers of biological information and function**
- ▶ “DNA encodes the genetic information that is passed on to RNA, which acts as the messenger for the synthesis of proteins. **Protein** enzymatic function can then give rise to a plethora of **structurally diverse small molecules**. In many cases, these molecules are the primary effectors of biological functions, acting at the DNA, RNA, and protein levels.”

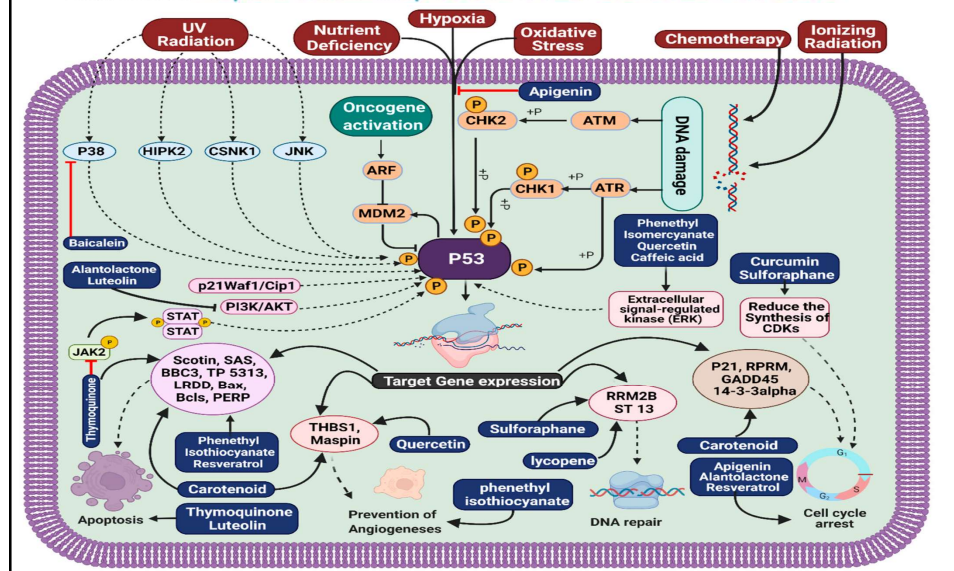
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SMALL MOLECULES

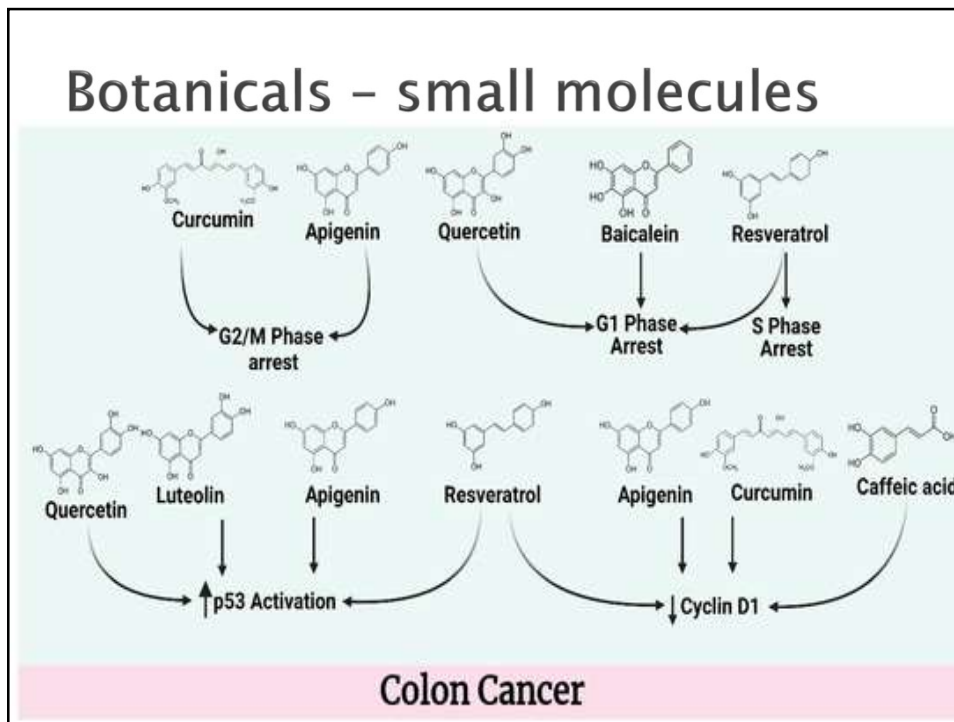
- ▶ “Although both DNA and RNA have central functions in the maintenance and decoding of genetic information, the real effectors of these functions are proteins. In the case of **structural proteins, they represent the end of the road for a given biological property or function.** However, for the majority of proteins, **CATALYTIC ACTIVITY** is the main function, thus extending their biological properties to the products of the reactions catalyzed: a plethora of structurally diverse small molecules. It is, therefore, these **SMALL MOLECULES** that **constitute the *raison d’être* of biological function in most cases.** Without identifying and studying these molecules, we will not fully understand the functions of metabolic pathways and the interconnections between them. Nor will we be able to fully comprehend the complexities of any biological system. We now have the tools to delve into the unexplored sources of many intriguing molecules in our own bodies. This should be done not only with an intellectual view toward understanding the molecular intricacies of life in more detail but also with a practical view of benefiting from what these molecules may have to offer.”

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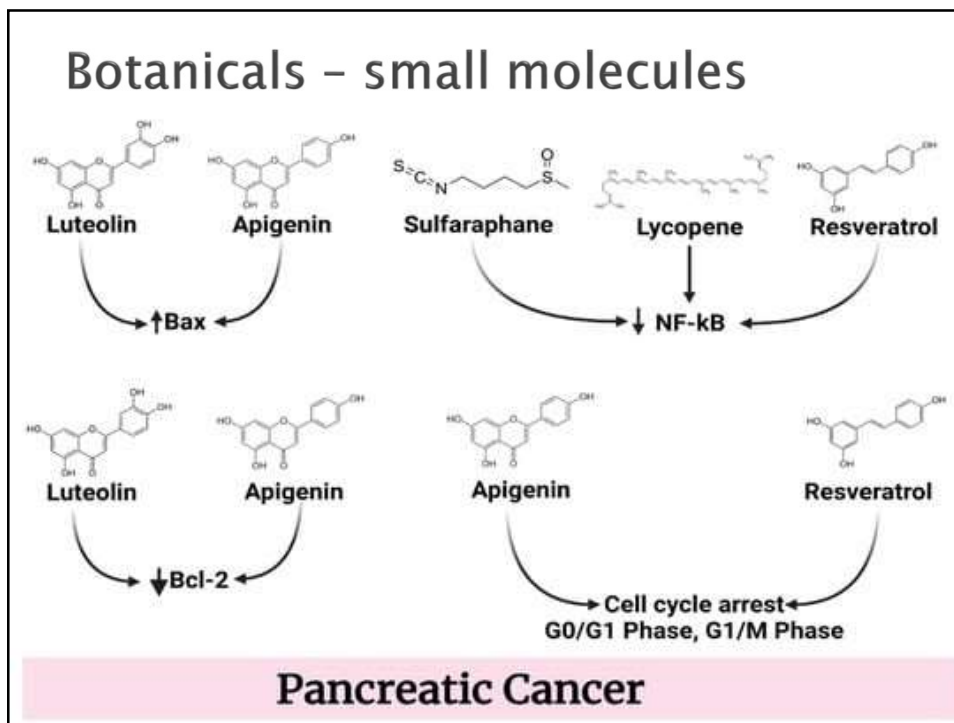
Illustration representing the probable blockade of sites by small molecules in the gastrointestinal tract and associated cancers. <https://www.mdpi.com/1420-3049/27/17/5686>



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GUT–BRAIN AXIS (GBA)

- ▶ Complex BIDIRECTIONAL communicative and regulatory system involving the brain and central nervous system and the enteric environment of the gut.
- ▶ “The enteric nervous system doesn’t seem capable of thought as we know it, but it communicates back and forth with our big brain — with profound results. For decades, researchers and doctors thought that anxiety and depression contributed to these problems. But our studies and others show that it may also be the other way around,”
 - Jay Pasricha, M.D., director, Johns Hopkins Center for Neurogastroenterology,
- ▶ Irritation in the gastrointestinal system may be sending signals to the central nervous system (CNS) that trigger mood changes.

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MOLECULES OF EMOTION – Candace Pert – 1997

- ▶ Georgetown Medical Center in Washington
- ▶ Peptides that flood our bodies are, in fact, the molecules of emotion.
- ▶ Emotions, largely ignored within the traditional confines of science and medicine, are actually the key to understanding psychoimmunology's emerging picture of how body and mind affect each other.

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MOLECULES OF EMOTION

- ▶ For Pert the body’s “information system” has two major elements—the chemical substances known as neuropeptides and the receptors into which they fit. Neuropeptides are produced by nerve cells in the brain, and when they lock into their receptors, which are attached to other cells in the body, they make something happen (or prevent it from happening).
- ▶ Pert outlines a new view of the body’s internal conversation, a conversation that appears to be remarkably flexible, varied, and subtle. For example, certain immune cells carry all the receptors identified so far, which means, presumably, that they can be affected by a wide range of “messages.”

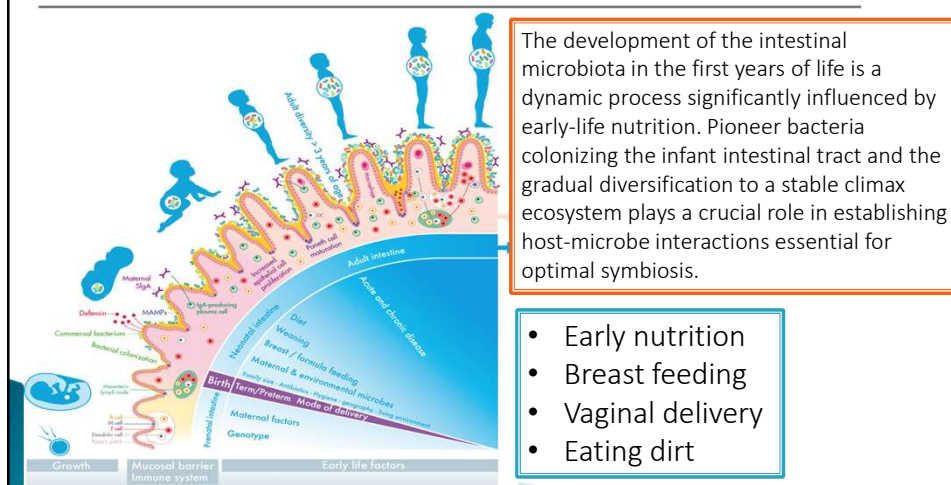
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Shaping the Human Metabolome

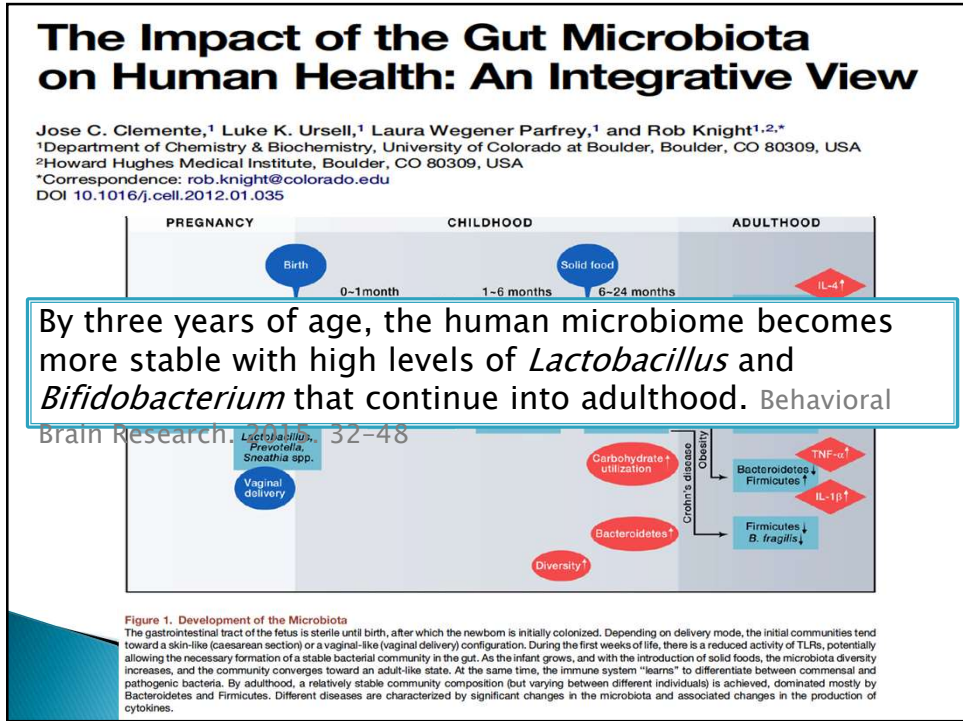
The first thousand days – intestinal microbiology of early life: establishing a symbiosis

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Pediatr Allergy Immunol 2008; 19: 682-687
 DOI: 10.1111/j.1399-3038.2008.00731.x

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PEDIATRIC ALLERGY AND IMMUNOLOGY

Review Article

Is caesarean delivery associated with sensitization to food allergens and IgE-mediated food allergy: A systematic review

Review study about n=15,000 children
 Age: 2-10

This systematic review found evidence that children delivered by caesarean section have an increased rate of sensitization to food allergens compared with those delivered by vaginal birth. In addition, there is evidence from one study that symptoms of food allergy occur more commonly among children who are born by caesarean section

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GI Microbial Colonization & Development

- ▶ GI colonization begins in utero
 - Once thought to be a sterile interaction, placental and amniotic microbes may be the first influence on the fetus The Placenta Harbors a Unique Microbiome. Science Translational Medicine 21 May 2014: Vol. 6, Issue 237, pp. 237ra65, Clin Infect Dis. 1997 Jun; 24(6):1228-32.
 - Birthing methods, breast milk/formula and interaction with environment will have greatest impact on microbial diversity and numbers
- ▶ GI health has an intimate relationship with proper immune responses; up-regulation and down-regulation
 - i.e: GI health related to autoimmunity and immunodeficiency

Bifidobacterium and Lactobacillus DNA in the human placenta. Satokari R, Grönroos T, Laitinen K, Salminen S, Isolauri E. Lett Appl Microbiol. 2009 Jan; 48(1):8-12.
Regulation of T cell responses in the developing human fetus. Michaëlsson J, Mold JE, McCune JM, Nixon DF. J Immunol. 2006 May 15; 176(10):5741-8.
T cell subclasses in T cells of human ileum. Spencer J, Dillon SB, Isaacson PG, MacDonald TT. Clin Exp Immunol. 1986 Sep; 65(3):553-8.

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Meet the Other Players

- ▶ There are also resident viruses, which consist mostly of bacteriophages. This is called your **virome**. Bacteriophages are viruses that infect bacteria in a highly specific manner. These bacteriophages play a role in the development of the microbiome as well as pressure bias towards one group of bacteria or another

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Antibiotics and the Microbiome

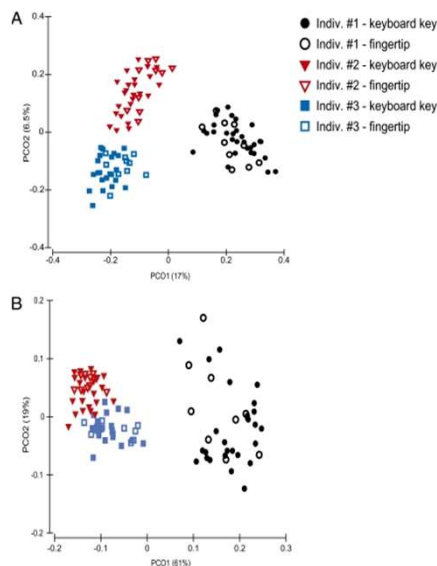
Even just a single dose of antibiotics can disrupt the microbiome and may take up to a year to recover. Sustained use of antibiotics may lead to permanent changes and a negative dysbiosis within the human GI microbiome

Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci USA*. 2011;108(1):4554-4561.
 Relman DA. The human microbiome: ecosystem resilience and health. *Nutr Rev*. 2012;70(Suppl. 1):2-9.

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The Microbiome

- ▶ You are unique but it may not be your DNA that makes you stand out...
 - 99.99% of your genome is identical to your neighbor yet you may only share 10% similarity in your microbiome makeup
 - Up to 90% accuracy scientists can match you to the mouse/keyboard you use at work *Proc Natl Acad Sci U S A*. 2010 Apr 6. 107(14): 6477-6481.



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Gastrointestinal: Definitions

Microbiota– collection or community of microbes


↓

Microbiome– microbiota plus their genetic material

↓

Metabolome– microbiome plus it's collective metabolites

↓



<http://learn.genetics.utah.edu/content/microbiology/>

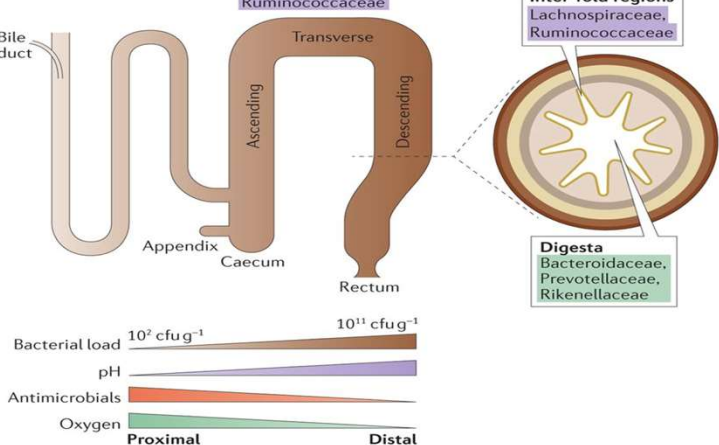
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Gut Bacteria and Locations

Dominant gut phyla:
Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia

Predominant families in the:

<p>Small intestine</p> <ul style="list-style-type: none"> Lactobacillaceae, Enterobacteriaceae 	<p>Colon</p> <ul style="list-style-type: none"> Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae, Ruminococcaceae 	<p>Inter-fold regions</p> <ul style="list-style-type: none"> Lachnospiraceae, Ruminococcaceae
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Bacterial load: 10^2 cfug⁻¹ (proximal) to 10^{11} cfug⁻¹ (distal)

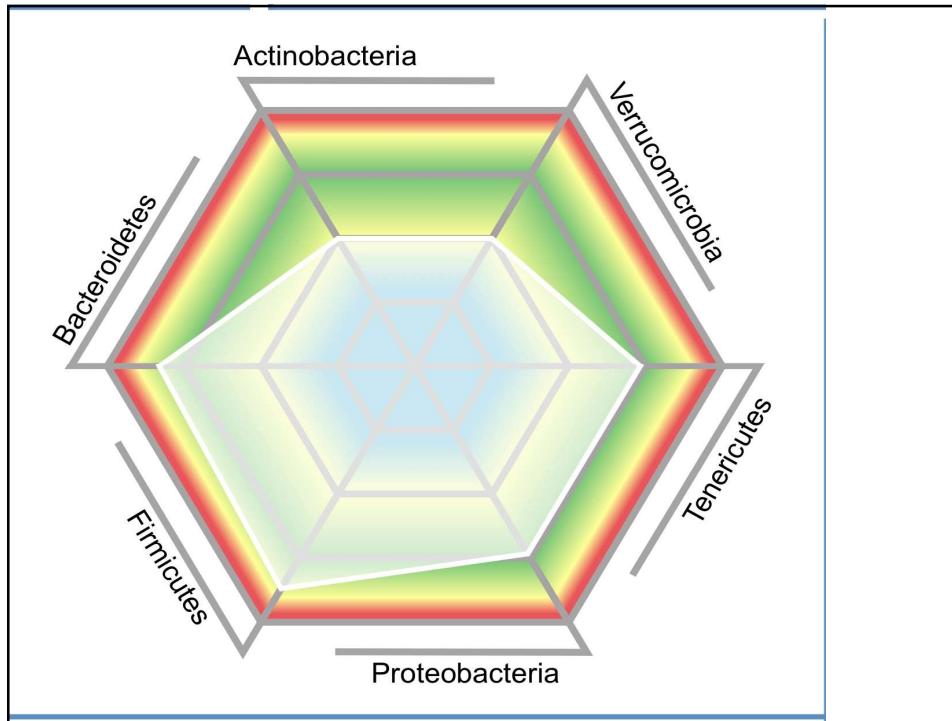
pH: (decreases from proximal to distal)

Antimicrobials: (decreases from proximal to distal)

Oxygen: (decreases from proximal to distal)

Nature Reviews Microbiology volume 14, pages 20-32 (2016) Nature Reviews | Microbiology

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Not One Size Fits All

There is a high amount of individuality that needs to be accounted for when interpreting the microbiome

- There is no “one size fits all” population ratio
- Definition of a healthy microbiome- ideal collection of genes and pathways (healthy metabolic functions) rather than specific populations
- Survey of 4788 samples from 242 “healthy” adults noted fecal bacteria populations were varied yet metabolic pathways conserved
 - “Microbiome fingerprint”

Defining a healthy human gut microbiome: current concepts, future directions and clinical applications. Cell Host Microbe. 2012 Nov 15;12(5):611–22

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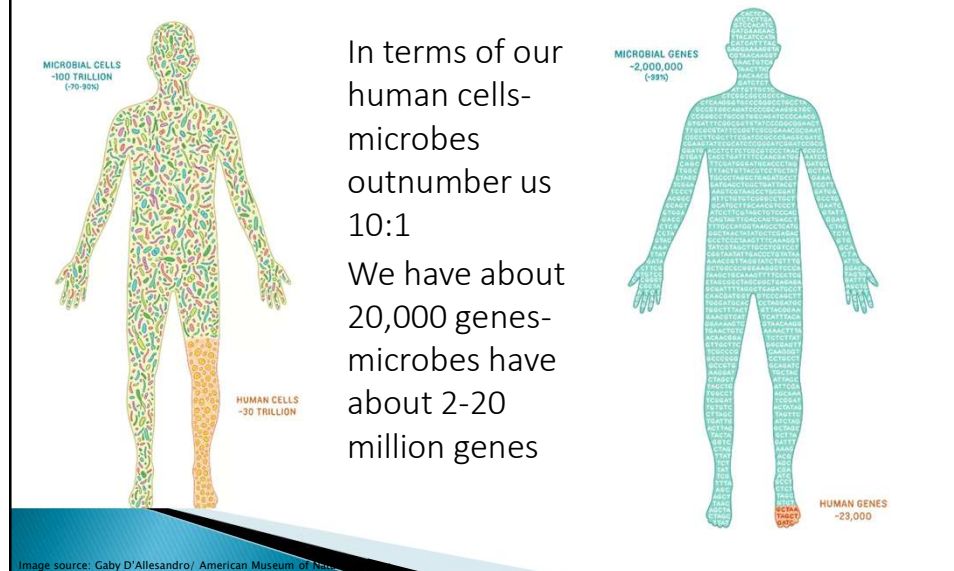
The Human Ecosystem

Most scientists are now alluding to our resident **microbes** as being a **SEPARATE ORGAN**

- Microbes are found
 - Skin (e.g. Staphylococcus aureus)
 - Mouth (e.g. Streptococci)
 - Stomach (e.g. H. pylori)
 - Intestines(e.g. lactics, enterics, enterococci, bifidobacteria)
 - Also contains low populations of pathogenic bacteria C. difficile that remain asymptomatic until the beneficial flora is reduced
 - Urogenital (e.g. staphylococci, corynebacteria, enterics)
 - Vagina (e.g. lactobacilli)
 - Nasal cavity (e.g. staphylococci and corynebacteria)

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You are Outnumbered



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