Abstract

Food Quality Control:
Nutrition and Immunity in Intestinal Homeostasis

Zuri Ayana Sullivan

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The gastrointestinal (GI) tract is a multi-kingdom cellular ecosystem that facilitates the procurement of nutrients from the environment. In constant contact with the external world, it is at once a point of exposure to lethal pathogens and toxins, and the locus of absorption for nutrients that are essential for life. Consequently, this tissue is tasked with the challenge of balancing its primary functions of nutrient uptake and host defense in response to a complex and constantly changing environment.

This challenge is particularly significant for omnivores, whose diets change on daily, seasonal, and lifelong timescales, alongside encounters with ingested toxins, enteric pathogens, and commensal microbes. Omnivorous lifestyles therefore require that the GI tract of such animals be adaptable to the dynamic nature of their environments. This dissertation explores the cellular and molecular mechanisms that confer this adaptability.

Chapter 1 provides an ecological perspective on the physiology of the gastrointestinal tract, and defines the major cellular players in nutrient uptake and host defense in this tissue. Chapter 2 presents a conceptual framework for understanding the basis of immunological responses to food, and describes how systems that monitor the quality of food become exaggerated in allergic disease. Chapter 3 explores the intestinal epithelial response to

nutrient sensing, and provides evidence that diet can alter the cellular composition of the intestinal epithelium. Chapter 4 details the contribution of a poorly understood subset of intestinal lymphocytes, γδ T-cells, to the on-demand induction of digestive and absorptive machinery in response to diet. Chapter 5 examines mechanisms by which hosts may monitor the state of their commensal microbiota, using diet as a tool to perturb the microbial ecosystem. Finally, in Chapter 6, I propose a set of principles to explain how the intestine adjusts the balance between nutrient uptake and host defense in response to environmental change.

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Zuri Ayana Sullivan

Dissertation Director: Ruslan Medzhitov, Ph.D.

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Dedication

I dedicate this dissertation to the activists and trailblazers, thought leaders and freedom fighters, and all others, past and present, working to bend the arc of the moral universe towards justice.

Your struggle is my inspiration.

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List of Abbreviations

ABC	ATP-binding cassette
AgRP	agouti-related peptide
AHR	aryl hydrocarbon
BTNL	butyrophilin-like
CBC	crypt base columnar cell
CCK	Cholecystokinin
cDC	classical dendritic cell
CGRP	calcitonin gene-related peptide
CNS	central nervous system
COX	cyclooxygenase
CS	conditional stimulus
CTA	conditioned taste aversion
DAPI	4′,6-diamidino-2-phenylindole
DRG	dorsal root ganglion
EEC	enteroendocrine cell
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
EpCAM	Epithelial cell adhesion molecule
FABP	fatty acid binding protein
FQC	food quality control
GAP	goblet cell associated antigen passage
GI	gastrointestinal
GIP	gastric inhibitory peptide
GLP	glucagon like-peptide
GPCR	G protein-coupled receptor
HMO	human milk oligosaccharide
IEC	intestinal epithelial cell
IEL	intraepithelial lymphocyte
IFN	interferon
Ig	immunoglobulin
IGF	Insulin-like growth factor
IL	interleukin
ILC	innate lymphoid cell
IPA	ingenuity pathway analysis
ISC	intestinal stem cell
LP	lamina propria
LPS	lipopolysaccharide

LTP	lipid transport protein
M cell	microfold cell
MAIT	mucosal associated invariant T
MHC	major histocompatibility complex
MyD88	Myeloid differentiation primary response 88
NK	natural killer
NMU	neuromedin U
NOD	non-obese diabetic
NTS	nucleus tractus solitarius
OUT	operational taxonomic unit
PAF	platelet activating factor
PBN	parabrachial nucleus
Poly I:C	polyinosinic:polycytidylic acid
POMC	prooopiomelanocortin
PR	pathogenesis related
PRR	pattern recognition receptor
PSM	plant secondary metabolite
PUL	polysaccharide utilization loci
PXR	pregnane X receptor
ROR	RAR-related orphan receptor
SCFA	short chain fatty acid
scRNAseq	single-cell RNA sequencing
SFB	segmented filamentous bacteria
SGLT	sodium glucose transport protein
TA	transit amplifying
TCR	T-cell receptor
Tfh	follicular T helper
TGF	transforming growth factor
TLR	toll like receptor
Treg	regulatory T-cell
TSLP	thymic stromal lymphopoietin
tSNE	t-dependent stochastic neighbor embedding
UCS	unconditional stimulus
UR	unconditioned response
VANM	vancomycin ampicillin neomycin metronidazole
ZT	Zeitgeber time

Chapter 1: Introduction

The digestive tract is an ancient structure conserved across metazoans that facilitates the procurement of nutrients from the environment. In direct contact with the outside world, digestive tissues are also a site of exposure to pathogens and toxins, necessitating multilayered systems of host defense. The design and organization of digestive systems are highly diverse, mirroring diverse species ecology and nutritional strategies. In mammals, the gastrointestinal (GI) tract consists of several specialized organs that each contributes a specific step in the digestive process. The largest of these is the small intestine, which carries out the final steps in nutrient procurement, and is composed principally of a single-layer of absorptive and secretory epithelial cells that self-renews every 4-5 days (*1*). Intimately coupled with the intestinal epithelium are the largest population of immune cells of any organ in the body, the enormous sensory capacity of the enteric nervous system, sometimes referred to as the "second brain," and the trillions of commensal microbes whose constitutive colonization of the GI tract is a fundamental feature of mammalian physiology (*2-4*). The exquisite coordination of these diverse cell types balances the fortification of a barrier against potentially lethal environmental threats with the absorption of dietary nutrients that are essential for survival. This dissertation examines the cellular and molecular mechanisms that link nutrient uptake and host defense in the intestine.

Ecological physiology of the gastrointestinal tract

The logic underlying the organization and design features of digestive systems is best understood through the lens of evolutionary ecology. In (*5*), William H. Karasov and

colleagues propose three unifying principles for understanding the ecological physiology of these systems:

(1) *Variation in the nutritional value and chemical composition of foods drives diversification of digestive systems.*

Across species, the presence and number of enzymes and transporters for a given nutrient are positively correlated with the level of the corresponding nutrient in an animal's diet (*5*). For example, carnivores, whose diet consists primarily of protein and lipids, have lower rates of intestinal glucose uptake than other vertebrates, and changes in copy number of the epithelial glucose transporter SGLT1 mirror the relative abundance of carbohydrates across diets (*5*). Furthermore, copy number variation in amylase, which carries out enzymatic digestion of starch, is observed across primates – humans, whose diets are high in starch-rich storage polysaccharides, have more copies of salivary amylase than do chimpanzees or bonobos (*5*). Additional variation in the expression level of specific enzymes can be observed across populations within a given species. A familiar example is the preponderance of lactose intolerance across adult mammals, following the cessation of lactation and introduction of solid food, due to diminished expression of intestinal lactase upon weaning. In human populations historically associated with domestic ungulates (cows, sheep, and goats), however, polymorphisms in lactase regulatory regions confer an increased frequency of adult lactose tolerance (*6*).

In addition to variations in the number and expression level of digestive enzymes, animals also differ in the organization and morphology of their digestive systems in a manner that

reflects the nutritional composition of their respective diets (*5*). Herbivores, whose diets contain an abundance of structural carbohydrates refractory to digestion, generally have longer digestive tracts than carnivores of comparable body size (*5*). This length affords adequate time for the digestion of these refractory materials.

(2) *Simplified models clarify the complex array of digestive forms observed across metazoans.*

Digestive strategies can be summarized in three categories: (1) catalytic digestion, which relies on the activity of endogenous enzymes for nutrient harvesting; (2) phenotypic plasticity, in which digestive activity is adjusted to according to diet composition; (3) microbial fermentation, in which resident bacteria of the gut microbiota extend the enzymatic capacity of their host (*5*). Species differences in the relative utilization of each strategy accord with the chemical composition of different foods.

Penry and Jumars categorize digestive systems as belonging to one of three types of chemical reactors: batch reactors, such as the gastric cavity of hydra; plug flow reactors (PFRs) such as the tubular intestine of most vertebrates; and continuous flow stirred tank reactors (CSTRs), such as the rumen of cows (*7*). Each of these reactor types is optimized for different nutritional strategies. PFRs are best suited for digestion that involves catalytic activity of endogenous enzymes, while CSTRs maximize fermentation for those animals who rely heavily on the catalytic activity of microbial enzymes (*5, 7*).

Additional models help explain the relationship between different variable features of digestive systems. The efficiency of nutrient extraction, which confers positive fitness, is defined by Karasov and colleagues as follows (*5*):

$$Extraction\ efficiency \propto \frac{reaction\ rate \times\ digesta\ retention\ time}{concentration\ of\ reactants\ \times\ reactor\ volume}$$

This equation illustrates the relationship between nutrient extraction efficiency and the size of different digestive organs (e.g. reactor volume) versus diet (e.g. concentration of reactants), and formalizes the relationships between diverse digestive forms and diets across animals.

(3) *Digestive design accords with the "economy of nature."*

Digestive organs are energetically costly to maintain – in vertebrates, the digestive tract and liver can account for up to 25 % of total energy expenditure (*5*). Principles of economic design therefore help explain variation in digestive features across species (*5*). Accordingly, the size and performance of a given digestive system should be matched to the intake and nutrient density of its corresponding foods (*5*). In conjunction with the two principles outlined above, the "economy of nature" dictates that individual digestive features are designed to efficiently process a limited number of foods in accordance with species ecology (*5*). These features include the size and complexity of the digestive system, the elaboration of specialized digestive organs, the relative reliance on microbial fermentation, and the expression and number of enzymes and transporters for the breakdown and absorption of individual nutrients. A critical point is that, for humans and other omnivores, each of these features is adaptable to the availability and selection of

different foods, in accordance with the principles defined above. The mechanisms underlying the adaptability of each of these features are the focus of this dissertation.

In summary, the design of digestive systems is dictated by species ecology, with animals who consume specialized diets having particular features of the GI tract exaggerated to meet their nutritional needs. For generalists, animals that consume a wide variety of food sources, digestive systems are flexible, and enable adaptability to the shifting consumption of different nutrients from day to day and over the lifespan of the organism (Figure 1.1). The cellular and molecular mechanisms that confer this adaptability are incompletely understood. How and whether the mechanisms that confer efficiency in nutrient uptake interact with systems of host defense, the other major function of the GI tract, are largely unknown. I explore each of these questions in subsequent chapters in this dissertation. In the remainder of this Chapter, I review the major epithelial and hematopoietic cell types that contribute to nutrient uptake and host defense, and briefly discuss links between diet, immunity, and commensal microbiota.

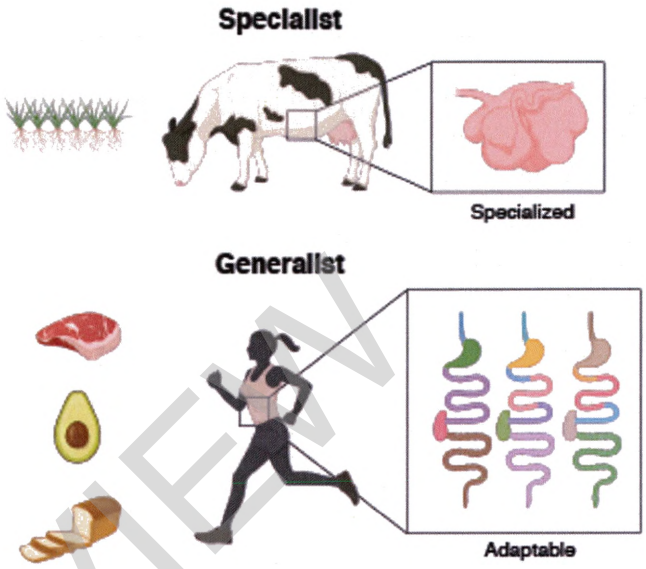

Figure 1.1: Digestive design reflects species ecology.

Intestinal epithelium

In mammals, the task of nutrient absorption is accomplished through the coordinated activity of multiple organs in the GI tract and can be summarized in three phases. In the cephalic phase, aroma, sight, smell, and taste of food trigger anticipatory responses along the length of the GI tract to prepare for the incoming food bolus (*8-10*). During the gastric phase, food is mechanically and chemically disrupted through the action of churning and gastric acid secretion in the stomach (*8, 11*). Finally, in the intestinal phase, the food bolus enters the duodenum where it is met with bile acids secreted from the gallbladder that aid in lipid emulsification, as well as pancreatic proteases, amylases, and lipases, which act on proteins, starch, and lipids, respectively (*8*). Following enzymatic digestion in the duodenum, macronutrient substrates are further digested by enzymes expressed on the brush border of absorptive enterocytes in the jejunum and proximal ileum, allowing for their final absorption via transporters expressed on the apical surface of absorptive epithelial cells called enterocytes (*8, 12*). Nutrients are subsequently secreted across the basolateral enterocyte membrane where they enter systemic metabolism (*8*). The small intestine epithelium therefore sits at the intersection of the external and internal environments, the site at which ingested nutrients are absorbed and distributed throughout the organism, and a principal line of defense against external threats.

The small intestine is organized into units called crypts, invaginations in the tissue that house intestinal stem cells at their base, and villi, protrusions into the lumen that are composed of differentiated mature epithelial cells (*13*). Crypts house the intestinal stem cell niche, which includes epithelial stem cells, Paneth cells, and mesenchymal cells.

Epithelial cells become more differentiated as they move up the crypt – villus axis, directed by signals that will be discussed later. Differentiated cells (with the exception of Paneth cells) migrate upwards towards the villus tip, and apoptotic cells are shed into the lumen, a process called annoikis, 3-5 days after their birth (*13*). It is likely that this lifespan may have exceptions for specialized secretory epithelial cells.

The small intestine epithelium is a highly dynamic tissue that self-renews every 4-5 days from a pool of constantly cycling epithelial stem cells called crypt base columnar (CBC) cells (*13*). These cells express the surface receptor Lgr5, and numerous genetic tools using this marker have enabled researchers to probe the biology of intestinal stem cells with precision (*1*). The rapid self-renewal of the intestinal epithelium is understood to enable its resilience to damage by environmental insults and the digestive process (*13*). In Chapter 6, I propose that in addition to protecting the tissue from environmental insults, rapid self-renewal allows for plasticity in the cellular composition of the epithelium itself, thereby enabling *adaptability* to the constantly shifting environment.

Intestinal epithelial cells (IECs) can be divided into two main lineages: absorptive and secretory. Absorptive cells include enterocytes and microfold (M) cells, and the secretory lineage includes enteroendocrine cells (EECs), goblet cells, Paneth cells, and tuft cells (*13*). The self-renewal and lineage specification of intestinal epithelial cells are controlled by key signaling pathways, most notably Wnt, Notch, EGF, and BMP. Wnt ligands are expressed in a gradient from the crypt, where they are most highly expressed, to the tip of the villus (*1, 14*). Wnt signaling through the receptor complex including Frizzled, Lpr5/6

on ISCs controls self-renewal of ISCs (*13*). BMPs exist in a reverse gradient to Wnt ligands, reaching their highest concentration at the villus tip, and promote differentiation over proliferation of intestinal stem cells (*13*). EGF signaling via ligands EGF and TGFα controls the proliferation rate of ISCs (*13*). Finally, Notch signaling by ligands DLL1 and DLL4 controls the fate choice between absorptive and secretory lineages (*13*). Notch signaling blocks the differentiation of secretory cells and maintains the ratio of absorptive to secretory cells in the epithelium (*13*).

Enterocytes

Approximately 80% of the intestinal epithelium is composed of absorptive enterocytes (*13*). These cells express brush border enzymes and nutrient transporters that carry out the digestion and uptake of dietary nutrients (*8*). Specifically, enterocytes express oligosaccharidases, peptidasees, and fatty acid binding proteins that carry out the final steps in digestion of polysaccharides, proteins, and lipids, respectively (*8*). Importantly, digestion of lipids involves the formation of chylomicrons within enterocytes that are further secreted across the basolateral membrane, following which they enter the lymphatics (*8*). Monosaccharides, peptides, and amino acids are absorbed across the apical membrane of enterocytes by specialized transporters, and enter portal circulation following transport across the basolateral membrane (*8*). The degree to which brush border enzymes and transporters are expressed heterogeneously across enterocyte, and whether enterocytes can be specialized for the absorption of different nutrients, are currently unknown.

Microfold cells

Microfold (M) cells are a specialized subset of absorptive epithelial cells that are located on top of Peyer's Patches, secondary lymphoid structures distributed along the length of the small intestine (*13*). M cells transport luminal antigen to immune cells within the Peyer's Patches, which gives rise to oral tolerance (*15*). M cell development is driven by RANK ligand produced by stromal cells in the Peyer's Patch (*13*).

Paneth cells

Paneth cells are secretory epithelial cells that reside at the base of small intestine crypts. Along with underlying mesenchymal cells, Paneth cells constitute the niche for intestinal stem cells (*13*). Unlike other mature intestinal epithelial cells, Paneth cells migrate downward after differentiation, a process mediated by ephrinB2 and ephrinB3 (*13*). Within the base of the crypts, Paneth cells sit adjacent to CBCs in alternating units. Here, they produce Wnt ligands, EGF, and Notch signals that maintain the intestinal stem cell pool (*13*). In addition to their role in the maintenance of intestinal stem cells, Paneth cells secrete antimicrobial compounds including defensins, phospholipase-A2, lysozyme, and the antimicrobial lectins Reg3β and Reg3γ (*16, 17*). These compounds protect CBCs from microbial attack, and are also secreted into the lumen where they constitute an antimicrobial barrier, in conjunction with the mucus layer (*18*). Paneth cells have a longer lifespan than other mature epithelial cells, surviving for 1-2 months after their migration to the crypt base (*13*). Paneth cell differentiation depends on expression of the Wnt target gene Sox9, as well as MAPK signaling (*13*).

Goblet cells

Goblet cells are found at the highest frequency of all secretory epithelial cell types (*13*). Their differentiation depends on the transcription factor SPDEF, and hyperplasia can be driven by the type-2 immunity cytokines IL-4 and 13 (*19*). The principal role of goblet cell is secretion of mucins from secretory granules, the most abundant of which is MUC2 (*20*). Mucins are highly glycosylated proteins that form a gel-like matrix at that constitute a physical barrier against microbes in the lumen (*20*). Microbial signals are required for proper development of the mucus barrier, and mucus glycans form a metabolic niche for specialized mucinophilic commensal organisms (*21, 22*). The small intestine contains a single mucus layer, while the more heavily colonized colon has a largely sterile, inner mucus layer, and an outer layer that is colonized by certain commensal microbes (*23*). In addition to their function in barrier defense through the secretion of mucus, goblet cells have been reported to play an important role in the uptake of luminal antigens and subsequent induction of oral tolerance through the activity of goblet cell associated antigen passages (GAPs) (*24*).

Tuft cells

Tuft cells are rare, isolated, chemosensory epithelial cells found at mucosal surfaces. In the small intestine, they constitute <0.4% of the epithelium (*13*). Named for the microtubule-rich cluster of microvilli that form a "tuft" on their apical membrane, they were first identified over 50 years ago, and only in recent years has their biology been defined in molecular detail (*25*). Tuft cells are now defined as secretory epithelial cells that express