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Spatial transcriptomics: paving the way for tissue-level systems biology

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The tissues in our bodies are complex systems composed of diverse cell types that often interact in highly structured repeating anatomical units. External gradients of morphogens, directional blood flow, as well as the secretion and absorption of materials by cells generate distinct microenvironments at different tissue coordinates. Such spatial heterogeneity enables optimized function through division of labor among cells. Unraveling the design principles that govern this spatial division of labor requires techniques to quantify the entire transcriptomes of cells while accounting for their spatial coordinates. In this review we describe how recent advances in spatial transcriptomics open the way for tissue-level systems biology.

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Introduction

The field of Systems Biology has made a profound impact on our ability to reverse engineer the biological networks that govern cellular behavior. Fueled by biotechnological developments over the past two decades, researchers have been able to obtain a detailed description of regulatory, signaling and metabolic networks [1–3]. Remarkably, many of these networks exhibited features that were common to engineered systems, such as modularity, robustness and recurring building blocks [4]. The techniques used for these studies required 'bulk' analyses of extracts from many cells, be it RNA [5,6], proteins [7] or chromatin [8]. While highly informative for unicellular organisms or cultured cells, these techniques provide only partial information when the biological systems are heterogeneous.

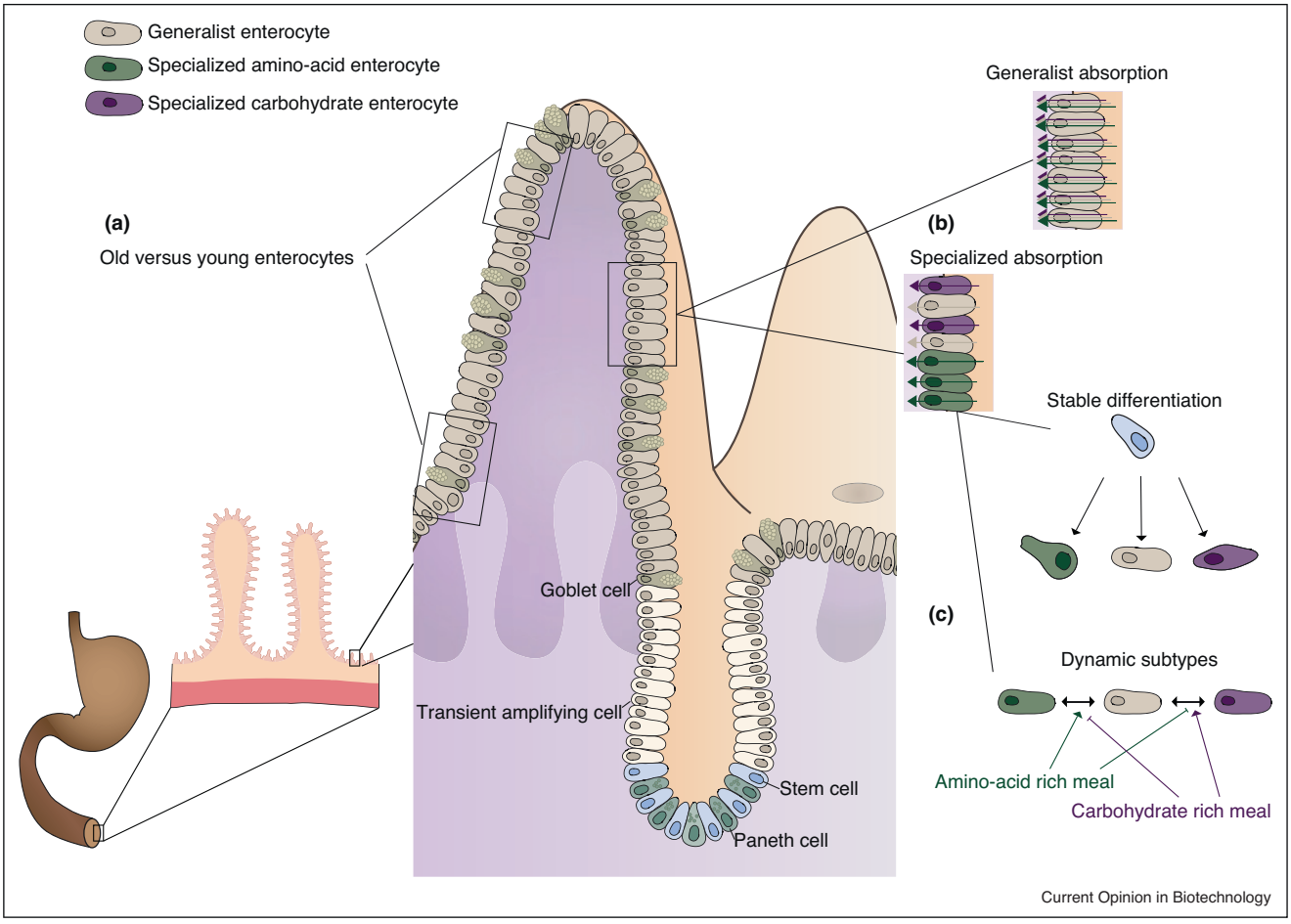
The tissues in our bodies consist of diverse cell types and subpopulations; their molecular identities would be lost in bulk measurements. Single cell RNA sequencing [9–13] has revolutionized our ability to identify and characterize such subpopulations. However, these techniques require tissue dissociation, thus losing the original tissue coordinates. Since the physical location of a cell within the tissue is a key determinant of its molecular identity, tissue-level systems biology requires obtaining whole-genome measurements while accounting for the spatial localization of cells. Several methods for spatial transcriptomics have been comprehensively reviewed in [14], herein we will focus on more recent advances in the field and their potential use in advancing our understanding of design principles of tissue organization.

Spatial context is a key determinant of cellular identity in mammalian tissues

To motivate the importance of spatial transcriptomics, we will shortly describe open questions in two typical heterogeneous, yet structured mammalian tissues—the intestine and the liver. The mammalian small intestine is lined with a highly folded epithelial sheet composed of deep pits called crypts, and larger protrusions called villi (Figure 1). Stem cells and progenitors within the crypt constantly feed the villus with secretory goblet cells and absorptive enterocytes. These differentiated cells rapidly migrate along the villus walls as they operate for a few days, until they are shed off from the villi tips. The positions of cells along the crypt villus axis correlate with their age, making this a classic system to study processes of cell differentiation, homeostasis, aging and death.

Extensive studies deciphered the homeostatic mechanisms that operate in the crypts to maintain constant stem cell numbers while ensuring a steady flux of differentiated cells [15–17]. Much less is known about the diverse processes in the differentiated compartments—the intestinal villi. How fast do enterocytes mature upon entering the villi? Do 'old' enterocytes at the villi tips operate less efficiently than 'young' enterocytes at the villi bottoms as a result of accumulated cellular damage acquired due to the hostile lumen environment (Figure 1a)? Are there different subtypes of enterocytes that subspecialize in the absorption of particular nutrients, such as carbohydrates, lipids or amino acids, or are enterocytes all-absorbing generalists (Figure 1b)? If such enterocyte division of labor exists is it a result of clonal subtypes generated in the crypts, or rather a transient division of labor modulated by the constantly changing

Figure 1



Open questions in the biology of the intestinal epithelium that require spatially resolved single-cell measurements. This illustration depicts a small intestinal crypt-villus unit. Each crypt forms a well-protected adult stem cell niche. Stem cells at the bottom of the crypt, intermingled with supporting Paneth cells, constantly proliferate to generate progenitors, termed 'transit amplifying cells'. These migrate upwards as they continue to divide yielding a constant flux of differentiated secretory goblet cells and nutrient absorbing enterocytes that feed the villi. The differentiated cells migrate upwards along the villi walls as they function for a few days until they are shed off from the villi tops. Several open questions regarding the collective behavior of this key cell population include: **(a)** Are 'old' enterocytes, that have arrived at the tip of the villus, functionally different from 'young' enterocytes that only recently acquired their differentiated function? **(b)** Are enterocytes 'generalists' that absorb each nutrient class equally efficiently or are there subspecialized types of enterocytes that preferably absorb carbohydrates, lipids or amino acids? **(c)** If such subspecialized enterocytes exist, do they differentiate hierarchically from stem cells and maintain their fate in a stable manner? Alternatively, the fractions of these subspecialized cells might fluctuate dynamically depending on extrinsic stimuli, for example, the presence or absence of the respective nutrients. Quantitative measurements of the complete transcriptome of enterocytes at defined coordinates along the villus axis can address these and other open questions.

nutrient composition in the gut (Figure 1c)? Are there spatial domains along the vertical villus axis for such subpopulations that define a hierarchy of absorption? Tools to characterize the complete cellular gene expression signatures of enterocytes along the vertical crypt-villus axis would address these open questions and unravel how the intestine can economically yet efficiently absorb nutrients that only exist for transient periods of time [18].

The mammalian liver is a second example in which the spatial locations of cells are critical for understanding their molecular identities and physiological roles. The liver is a central organ for maintaining organismal homeostasis. Hepatocytes perform a wealth of biological tasks including protein secretion, nutrient storage and release and detoxification. The hepatocytes that perform these tasks operate in repeating hexagonal anatomical units termed 'lobules'. Each lobule consists of around 12–15 concentric layers of hepatocytes and is polarized by blood that flows inward from outer portal nodes toward draining central veins (Figure 2). The absorption and secretion of hepatocytes residing along the radial blood vessels modulates the microenvironment available for more 'downstream'