Metanascentome: A genomic methodology proposal for the study of nascent metatranscription of the microbiota

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Abstract

The gut microbiota has been shown to have an important influence on host health. It has been reported that microbiota composition of each individual is stable across the adult life, but it may varies between individuals. Moreover, human gut microbiota composition differs across geography, according to host genetics, dietary habits, age, ethnic origin, geographic location and lifestyle. Nevertheless, gene composition or functional capacity is highly conserved across individuals, phenomenon known as functional redundancy. Although metatranscriptomics have the potential of study the mature mRNA from a microbiome sample, it is not easy to identify which bacteria is actively transcribing the genes who drives the molecular expression. The use of genome-wide methodologies to study the active mRNA synthesis seem to be useful to identify the bacterias who drives gene expression in microbiota environment.

Introduction

During recent years, the study of the human microbiome, especially the intestine, has focused the attention of various research groups given its relationship with different aspects of human health (Guinane & Cotter, 2013; De Clercq et al., 2017; Johnson et al., 2017). However, an important number of the studies that have been carried out to date have been limited to describing the different scenarios

in which the intestinal microbiota is altered (a phenomenon known as dysbiosis). Some researchers have studied the microbiota as if it were an organ (). But this is not totally correct, because microbiota have a dynamic changes across host life, from child development to eld. This means that it does not demonstrate typical organ-system biology. Little or nothing is known about the molecular mechanisms of gene expression involved in the changes of microbiota during host life. Even more, there is no background about how microbial gene expression is involved in the development and establishment of the altered microbiota (dysbiosis). This is mainly because the current methodologies for the study of the microbiota are merely descriptive, focusing on the massive sequencing of DNA or RNA: while massive sequencing of DNA provides information about taxa and functional pathways (or genes) that potentially participate in certain events, messenger RNA sequencing indicates which genes potentially will be expressed as protein (Simon & Daniel, 2011; Lavelle & Sokol, 2018). But, the latter is not entirely correct, since it does not take into account the dynamics and kinetics that RNA undergo through its existence (Pérez-Ortín, 2007; Pérez-Ortín et al., 2013b, a).

The amount of RNA present in a cell depends on two opposite processes, but related: the synthesis of RNA (transcription) and its degradation (Pérez-Ortín, 2007). This implies that messenger RNA (mRNA) quantification by traditional techniques, such as qRT-PCR or RNA-Seq, may be the result of the synthesis and degradation of mRNA is being quantified, which does not necessarily correspond to the RNA synthesis (or gene expression), at a given time. As well as all biological molecules, mRNA has a half-life, that correspond to a time in which it is a whole useful molecule (Pérez-Ortín et al., 2013b). Such a concept is known as stability, and is the result of the balance between synthesis and degradation of RNA. According to their nature, different RNAs have different half-lives. Therefore, microbiota RNAseq experiments (also known as metatranscriptomic), it is likely that only a biased part of the population is being quantified, capturing just the more stable mRNAs, but not necessarily the genes that are being transcribed or expressed at given time.

To understand the gene expression, it is necessary to use tools that allow the study of nascent mRNA dynamics at the genomic scale (Pérez-Ortín et al., 2013b). In the literature, several methods for the study of mRNA stability are described (Lugowski, Nicholson & Rissland, 2018). One of the first published genome-scale techniques for the study of nascent mRNA was the *Genomic Run On*, which is based on the incorporation of radioactively labeled UTP nucleotide to the nacent mRNA molecule that is being synthesized (García-Martinez, Aranda & Pérez-Ortín, 2004). Furthermore, this technique allows the determination of the quantity and the estimation of stability of RNA. Another example of the use of labeled nucleotide is the cDTA technique, which, by incorporating thiolated nucleotides, allows

the separation and identification of nascent RNA (Miller et al., 2011; Sun et al., 2012). Several other omic techniques developed for the study of nascent RNA are based on the co-immuno precipitation of the RNA-protein-DNA complex that forms the elongated RNA polymerase with the nascent RNA during gene transcription, or the immuno-precipitation of active RNA polymerase using specific antibodies (Pelechano et al., 2009; Churchman & Weissman, 2012). After capturing the nascent RNA molecule, it is possible to identify which gene corresponds by massive sequencing (Rhee & Pugh, 2012). Recently,

Despite the differences in the composition and abundance of microorganisms between different states of the human microbiome, there is an important functional redundancy, showing no large differences in the set of genes and metabolic pathways (Lozupone et al., 2012). It is possible that the origin in the development of different types of dysbiosis are due in part to the expression of specific group of genes belonging to microorganisms involved in the development and establishment of such bacterial imbalance. Identify which microorganism is expressing a given gene in certain conditions will allow to propose strategies for the diagnosis and treatment of several diseases whose bases are related to dysbiosis (such as obesity, intestinal inflammatory diseases or metabolic syndromes, and even in the development of some types of cancer).

Proposal

The use of nacentome methodologies to study the microbiome, such as methodologies based on the co-immunoprecipitation and/or the incorporation of labeled nucleotide to study the gene expression of the human microbiome will identify which bacteria drive the gene expression and which groups of genes are actively transcribed in the microbiome at given time, despite the functional DNA redundancy. Studying the nascent transcriptome of the intestinal microbiome will give us information about how the different bacteria that make up the intestinal microbiome are contributing to the altered state during disbyosis. Nevertheless, studying the microorganisms of the digestive system has its challenges. Many of them are strict anaerobes, while others are impossible to isolate. Therefore, it is necessary to use equipment that simulates the environmental conditions of the digestive system. Systems in series of bioreactors have simulated the gastro-intestinal ecosystem in order to study the activity and microbial diversity in different conditions (Molly, Vande Woestyne & Verstraete, 1993, Takagi et al., 2016). Also, few studies has been carried using an anaerobic pH/agitation controlled bioreactor, to access how human gut microbiome react under different conditions (Medina et al., 2017, Pinto et al., 2017). This kind of systems could be useful to test methodologies that allows the study of nascent transcription in

small consortium of species of the gut microbiome subjected to different scenarios. Capture nascent molecules of mRNA or active polymerases that are associated with the genes that are being transcribed at given time (in short, metanacentome), will answer which bacteria drives microbiota gene expression.

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