

MicroEpiQ: contact with adult as a major factor in microbiome-associated epigenetic modifications in Japanese quail (*Coturnix japonica*)

The microorganisms that live in the gut environment influence many functions of the host organism. This is because they interact with the intestinal walls and secrete small molecules (metabolites) that are absorbed into the bloodstream. This way, the microbiota affects virtually every tissue in the host's body. The genetic information stored by the gut microbiome is called the microbiome, and the metabolites it produces are called the metabolome. Tissues and organs are made up of cells under the control of the genomic DNA present in the nucleus. DNA encodes information expressed as RNA and protein, and thus determines the composition and functioning of the organism. However, there are various mechanisms that control DNA expression which are called epigenetic modifiers. Among the various epigenetic modifiers, DNA modifications such as DNA methylation or the activity of non-coding RNA mediators such as microRNA (miRNA) are the most common. At the genome-wide scale, these epigenetic modifiers are called the epigenome. There is a relationship between the microbiome and the metabolome and epigenome, which is called the microbiome-metabolome-epigenome axis. Essentially, this means that any changes in the microbiome will bring about changes in the metabolome and epigenome. This relationship means that the change in microflora is associated with a change in the way the host's DNA is expressed and how its organism functions. The deeper consequences of epigenetic modifications are the stable interference with gene expression. This means that if modulation is at the epigenetic level, it can be inherited to a daughter cell (mitotically) or even to the next generation (meiotically). In this project we address the crosstalk between the microbiome and the epigenome using quail as a biological model. The motivation of the project is based on the concept that the perinatal contact of the newly hatched quails with the adult mimics the natural transfer of the maternal microbiota to the offspring. This way, the droppings left by the adult initiate the inoculation of the microbiota of the neonates. The current grant proposal is based on empirical evidence that the contact with hen leads to reprogramming of the intestinal microbiota and metabolites of the neonatal chicks. Based on this initial concept, we developed the hypothesis that the intestinal microbiota stimulated by the contact with the adult provides sufficient environmental cue to trigger epigenetic modifications. Hereby, the primary goal of this project is to present the proof of concept that the contact with adult is the major factor in microbiome-associated epigenetic modifications in Japanese quail (*Coturnix japonica*). The project starts with the animal study, which is based on three generations of the Japanese quail, stimulated upon hatching by the contact with the adult individual. The treatment is supposed to mimick the natural transfer of the adult-like microbiota from mother to offspring and provide potent stimuli for development of the intestinal microbiota and related responses from the host. The downstream analyses will include: (1) metagenomic study to barcode the intestinal bacteria based on 16s rRNA sequencing; (2) determination of the metabolites produced by the intestinal bacteria, detectable in intestinal digesta and in serum; (3) sequencing of the miRNA, which is one of the microbiome-dependent epigenetic modifiers affecting expression of many genes in the genome; (4) sequencing of the methylated (vs. unmethylated) parts of the DNA to determine which areas have been transcriptionally deactivated (vs. activated) upon microbiome stimulation. In conclusions, we will outline the relationship between the modifiers of the microbiome and epigenome in quails, and determine whether the epigenetic aspect attributed to the microbiota is discernible over one generation or can provide a more profound stimuli to quail epigenetics. The results based on quail can be extrapolated to other avian species.