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HOST-MICROBIOTA INTERACTIONS DURING AGEING

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SUMMARY

Host-associated microbial communities take part to several fundamental aspects of host biology, including development, nutrient absorption, immunity and pathogenesis. Throughout host life, microbes associated with external body surfaces vary in composition and function and can shift from commensal to pathogenic. However, whether these microbial communities can directly influence host ageing has remained elusive for a long time. Recent work in laboratory model organisms has revealed for the first time that commensal microbes can actively modulate host ageing and that microbial communities associated with young hosts can have a health-promoting action in middle-age individuals that leads to significant life span extension. These findings suggest new opportunities to identify systemic ageing-modulating mechanisms via the gut microbiota and will help design innovative anti-ageing interventions that could impact human health and ageing-associated diseases.

AGEING AND HOMEOSTASIS

Ageing is associated with pervasive functional decline and overall decrease in physiological fitness that lead to a progressive increase in the risk of disease and death. During ageing, homeostatic processes fail at multiple levels of biological complexity. DNA repair mechanisms become less efficient (*Vaidya* et al., 2014) and protein homeostasis, which ensures proteome stability, functionally declines at older age (*Morimoto* and *Cuervo*, 2009). Membrane and organelle composition, as well as cell physiology, undergoes dramatic changes during ageing. Ageing is typically associated with increased risk of uncontrolled cellular proliferation – leading to cancer (*Finkel* et al., 2007) – and to protective proliferation arrest – called cellular senescence (*Childs* et al., 2015). If on the one hand cellular senescence acutely reduces the risk of cancer, on the other hand it leads to inflammation and higher risk for disease and frailty (*Tchkonia* et al., 2013). Changes in the extracellular matrix composition, which is importantly involved in wound healing, stem cell functionality and longevity in experimental animal models (*Ewald* et al., 2015), additionally contribute to the age-dependent decline in organ and organism performance.

A combination of genetic and non-genetic factors affects the rate at which different organisms age and tune life expectancy in different species. Research done in model organisms has revealed key conserved molecular pathways, including the insulin-IGF1, the mTOR and AMPK pathways, which regulate ageing and life span across several species, from yeast to mammals (*Kenyon* et al., 1993; *Kapahi* et al., 2010; *Lapierre* and *Hansen*, 2012;

Weir et al., 2017). Environmental manipulations, including temperature changes, different diets and various forms of stress, also significantly impact ageing in several model organisms (*Liu* and *Walford*, 1966; *Weindruch* et al., 1986; *Libert* et al., 2007; *Ermolaeva* et al., 2013). Overall, ageing and life span are complex physiological outcomes of the genetically-encoded strategies that organisms evolved to maintain homeostasis throughout their lifetime in response to the continuous exposure to internal and external stimuli.

GUT MICROBIOTA CONTRIBUTE TO HOST PHYSIOLOGY

Complex microbial communities covering host surfaces occupy the interface between organisms and the external environment, from roots and leaves in plants to skin and mucosal surface including lungs and gut in animals. These microbial communities participate in a wide range of key biological processes, including nutrition, development (*Sommer* and *Backhed*, 2013; *Hill* et al., 2016), essential vitamin synthesis, metabolism (*Nicholson* et al., 2012), immune modulation (*Geva-Zatorsky* et al., 2017), defence against pathogens and disease. Host-associated microbiota constitute highly diverse and dynamic communities, able to modulate and buffer host physiology by promptly reacting to internal (e.g. hormonal and metabolic) and external (e.g. temperature, toxins, pathogens) stimuli. A large amount of circulating host metabolites in the host serum are synthesized by gut microbes (*Wikoff* et al., 2009), further supporting that host-associated microbiota are not passive passengers on host surfaces, but rather an integrative physiological partner that contributes to host homeostasis. Due to their essential contribution to virtually all host biological functions, it is reasonable to hypothesise that commensal microbes could be essential players during host ageing and could be targeted to design interventions aimed at modulating ageing and age-associated conditions.

GUT MICROBIOTA AND IMMUNE FUNCTION: A RECIPROCAL INFLUENCE

Mounting evidence indicates that the gut microbiota play a prominent role in shaping the immune system during development. In humans, new-borns acquire their initial resident gut microbiota from several sources, most prominently from the birth canal and from maternal skin during breast-feeding. These early-acquired bacterial consortia shape the structure of mature gut microbiota (*Dominguez-Bello* et al., 2010) and play an essential role for immunological development (*Round* and *Mazmanian*, 2009). Caesarean sections and formula feeding dramatically affect the gut microbial composition, and consequently impact proper immune system development, with potentially long-lasting effects for individual health (*Bokulich* et al., 2016). Gut bacteria interact with cells of both the innate and adaptive immune system in the gut mucosa and different metabolites synthesized by gut bacteria can induce pro or anti-inflammatory host responses (*Arpaia* and *Rudensky*, 2014). Epitopes derived from gut bacteria, both commensal and pathogenic, significantly shape the evolution of lymphocyte antibody and receptor

repertoires (*Zhao* and *Elson*, 2018). Consistently, specific-pathogen-free (SFP) raised mice have a much higher immunoglobulin (IgA) diversity compared to mono-colonized mice (*Lindner* et al., 2015).

While bacteria shape immune cells development and function, immune cells, in turn, play a key role in determining and controlling the composition and abundance of gut microbial communities. Pharmacologically immunosuppressed rats undergo a loss of commensal gut microbe diversity, which are generally associated with a healthy status (*Bhat* et al., 2017). Specific antibodies, i.e. IgAs, synthesized by intestinal plasma cells, are majorly secreted in the intestinal lumen and bind to a broad subset of bacteria to help maintain a healthy barrier function (*Bunker* et al., 2017). Under normal physiological conditions, IgAs target bacterial taxa that otherwise would contribute to pathogenesis (*Planer* et al., 2016). Conversely, specific pathogens, including influenza virus, succeed in infecting the host by precisely degrading human IgAs.

While bacteria influence immune system evolution, on the other hand the immune system acts as a selective force to shape the composition and function of gut microbes.

GUT MICROBIOTA IN AGEING AND DISEASE

Diet and lifestyle importantly affect differences in microbiota composition among individuals (*Rothschild* et al., 2018). The composition of host-associated gut microbiota undergoes dramatic changes in humans after birth, becomes stable during adulthood in non-pathological conditions, varies during pregnancy and then goes through drastic changes during ageing (*Kostic* et al., 2013). Although stable in composition during adulthood, microbiota has rapid functional metabolic oscillations during the day (*Thaiss* et al., 2016). Across many organisms, including laboratory flies, mice and humans, ageing is characterized by dramatic changes in the composition of the commensal gut microbiota, which could lead to dysbiosis and ultimately host demise (*Claesson* et al., 2012; *Guo* et al., 2014; *Clark* et al., 2015). While gut microbiota associated with healthy hosts are typically characterized by large bacterial taxonomic diversity, frailty and ageing are associated with loss of diversity and expansion of more pathogenic bacterial species. Studies across different human age cohorts have shown that large changes in the abundance of subdominant bacterial taxa in the gut are a hallmark of ageing. Moreover, exceptionally long-lived individuals, including supercentenarians, are characterized by the persistence of bacterial taxa associated with health (*Biagi* et al., 2016). While diversity-associated microbial taxa often decline during age, specific bacterial taxa, such as *Clostridiales*, are associated with malnutrition and increased frailty (*O'Toole* and *Jeffery*, 2015). In flies, reducing gut microbial dysbiosis by improving immune homeostasis promotes longer life span (*Guo* et al., 2015). In humans, after antibiotic treatment, pathogenic bacterial species, such as *Clostridium difficile* and *Enterococcus faecalis*, can restructure the gut microbial composition and cause severe chronic conditions that pose a major threat for public health (*Backhed* et al., 2012; *Milani* et al., 2016). In humans, faecal material transfer from healthy donors is successfully used in the clinic to resolve acute *Clostridium difficile* infections (*Lee* et al., 2016). Remarkably, transplanting microbes from obese individuals into

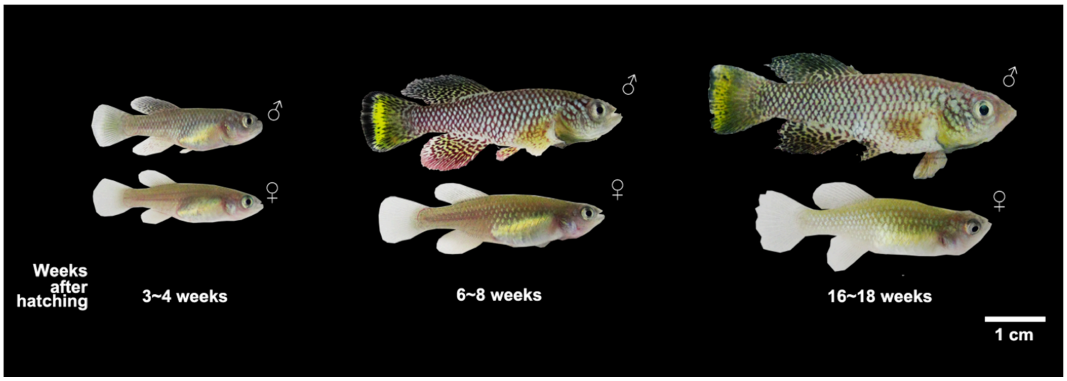

Figure 1: Ageing in the turquoise killifish. Reaching maturity as soon as 3-4 weeks post hatching, killifish display several ageing-related phenotypes by 16 weeks.

germ-free-raised mice leads to dramatic effects, including elevated adiposity and changes in fatty acid and amino acid metabolism, associated with systemic health (*Ridaura* et al., 2013). Overall, microbiota composition and function dramatically change during ageing and disease and manipulating the composition of the gut microbial communities via microbiota transplants has the opportunity to be a novel powerful intervention to impact the ageing process and improve systemic health.

GUT MICROBIOTA PLAY A CAUSAL ROLE IN MODULATING HOST AGEING AND LIFE SPAN

Despite evidence that the gut microbiota could dramatically modulate host metabolism and physiology, until recently it was not clear whether gut microbes could causally modulate host ageing and longevity. Recent work done in nematode worms, flies, fish and mice has shown that gut microbes can beneficially influence host ageing and life span, proving that specific components of this microbial consortium can *de facto* improve overall physiological fitness of the host (*Seidel* and *Valenzano*, 2018).

Work done in nematodes (*Caenorhabditis elegans*) has shown that worms feeding on different bacterial species and on varieties of *E. coli* strains different from the standard laboratory OP50 *E. coli* strain (*Girard* et al., 2007) live longer than standard-fed worms (*Sanchez-Blanco* et al., 2016; *Han* et al., 2017). A recent study in a short-lived fish has further shown in vertebrates that the gut microbiota is causally involved in modulating host ageing and life span, and that young-associated gut microbiota, transplanted to middle-age individuals, could lead to life span and health benefits (*Smith* et al., 2017). This study used the naturally short-lived turquoise killifish (*Nothobranchius furzeri*), which is the shortest-lived vertebrate raised in a laboratory setting (Figure 1), which includes captive strains with a median life span of about 4 months (*Cellerino* et al. 2016, *Valenzano* et al., 2017). For comparison, laboratory mice live 2.5–3 years and zebrafish can live until five years (*Kim* et al., 2016). Despite their short life span, turquoise killifish also display a wide range of age-related changes, including an increased occur-

rence of cancer, reduced regenerative capacity, increased cellular senescence, neurodegeneration and cognitive decline, making it a powerful new vertebrate model system to study ageing and age-related diseases (*Valenzano* et al., 2017). Turquoise killifish have complex gut microbiota, both in the wild and in captivity, similar in taxonomic diversity to mammals. During ageing, the overall microbial diversity of the resident gut microbiota decreases, while potentially pathogenic *Proteobacteria* become more prevalent, possibly contributing to host demise (*Smith* et al., 2017). After acute re-colonisation of the intestine of middle age individuals with gut microbiota from young donors, fish lived significantly longer, remained more active at old age, and kept highly diverse microbiota. Whether this gut microbial transfer influences immune function and whether its effect is sufficient to improve host health and therefore delay the ageing process is still an open question.

TURQUOISE KILLIFISH AS A MODEL TO STUDY VERTEBRATE IMMUNOSENESCENCE

Unlike invertebrate model organisms such as laboratory flies (*Drosophila*) and nematode worms (*Caenorhabditis elegans*), fish are equipped with an adaptive immune system consisting of both T and B-lymphocytes. B-lymphocytes undergo somatic recombination at the IgH (Immunoglobulin heavy chain) locus and generate an extremely diverse ($>10^9$) antibody repertoire, which enables to build complex immune responses towards extraneous agents, such as bacteria, viruses, etc. B-cells that bind antigens from microbes and viruses undergo amplification, affinity maturation and unfold a targeted immune response against them in a highly regulated process. Antibody repertoire diversity can be used as a proxy of the function of the whole B-lymphocyte compartment, with high diversity being better than low diversity, and antibody repertoire variation throughout ageing can be adopted as a biomarker of overall health status. Although it is known that upon ageing the B-cell repertoire declines (*Martin* et al., 2015), it is not clear whether this decline correlates with the decline in the gut microbial diversity. Currently, it is not clear what are the implications of the age-dependent decrease in immune function (e.g. decreased antibody repertoire diversity) for the changes in microbial diversity and function that occur during ageing. Age-dependent immune dysfunction could lead to proliferation of pathogenic bacteria that are already present in the young gut microbiota, eliciting bacterial community dynamics that favour more proliferative and pathogenic bacterial taxa over commensal and slow-replicating taxa. Alternatively, specific bacterial strains – within a specific subset of bacterial species – could evolve within the gut to escape immune attacks, independently of immune functional decline associated with ageing. Bacterial evolution towards increased pathogenicity could, in turn, lead to host damage and systemic functional decline. As a third alternative, age-dependent host immune dysfunction and bacterial evolution could occur simultaneously, leading to increased pathogenicity of gut bacteria and age-related diseases. The characterization of the sequence of the IgH locus in short-lived vertebrate species, such as the turquoise killifish, will enable to

study in detail whether the immunoglobulin diversity and abundance change during ageing and whether such changes are influenced by anti-ageing interventions, such as transplants of young-associated gut microbiota. Additionally, applying high-throughput approaches to study the immunoglobulin repertoire, such as the recently developed IgSeq method (*Weinstein* et al., 2009), will enable to functionally compare immunoglobulin diversity in the gut with commensal and pathogenic microbial diversity at different ages. High-resolution characterisation of immune system and microbiome changes during host life will lead to understand whether immune senescence precedes the loss of microbial diversity or whether age-dependent loss of microbial diversity in the gut anticipates immune senescence. The use of a short-lived and experimentally tractable vertebrate model organism, such as the turquoise killifish, will hence be instrumental to answer key questions more rapidly than in longer-lived vertebrate model organisms, such as zebrafish and mice.

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