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AGEING AND THE MICROBIOME – LESSONS FROM NON-SENESCENT MODELS

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SUMMARY

Research on the biological processes of ageing is at the brink of a revolution with respect to our understanding of its underlying mechanisms as well as our ability to prevent and cure a wide variety of age-related pathologies. Ageing is ordinarily recognized as a process that results from the combined influence of genetic and epigenetic determinants, life-style associated factors and external events. Studies using model organisms such as budding and fission yeasts, the nematode *Caenorhabditis elegans*, zebrafish and mice have generated significant insight into (epi)genetic pathways involved in ageing. However, developing an integrated understanding of these diverse processes and, thereby, achieving insight into the causes and mechanisms of the ageing process, remain a challenge. Here we review recent studies in non-senescent *Hydra* which demonstrate that a continuously high activity of the transcription factor FoxO contributes to continuous stem cell proliferation and at the same time also supports robust colonization of epithelia with a stable microbiome.

THE SIGNATURE OF AGEING

Numerous studies show that ageing is not a series of stochastic defects but a directed biological program that has a conserved genetic background (*Hitt* et al., 1999; *Kenyon*, 2010; *Jeck* et al., 2012; *López-Otín* et al., 2013) and is dependent on both genetic and environmental factors (*López-Otín* et al., 2013; *Dato* et al., 2017). Remarkably, only two proteins have been consistently associated with human ageing and longevity – Apolipoprotein E (Apo-E) and forkhead-box protein O3 (FoxO3). The genes were initially discovered in candidate gene studies of long-lived French (Apo-E) (*Schächter* et al., 1994) and long-lived Japanese/Okinawans in Hawaii (FoxO3) (*Willcox* et al., 2008), by comparing selected candidate gene frequency between long-lived cases (mainly centenarians) and shorter-lived controls. These findings were subsequently replicated in numerous populations worldwide (*Morris* et al., 2015). Genome-wide association studies (GWAS) utilizing larger numbers of cases and controls confirmed these genes as impacting human longevity but no other candidates have emerged with widespread replication (*Broer* et al., 2015). Apo-E plays an important role in clearance of cholesterol and lipoproteins from the circulatory system in vertebrates (*Phillips*, 2014). The gene consists of three alleles – the 'protective' allele ε2, the most common 'neutral' allele ε3 and the 'risk' allele ε4. While ε2 is associated with reduced risk for CVD and dementia as well as with increased life

span, ε4 contributes to opposite effects (*Schächter* et al., 1994; *Bennet* et al., 2007; *Nebel* et al., 2011; *Lindahl-Jacobsen* et al., 2013). FoxO is a conserved transcription factor that shuttles between cytoplasm and nucleus (*Van Der Heide* et al., 2004) and binds directly to the regulatory sequences of its target genes (*Webb* et al., 2016). Four mammalian members of the FoxO family are described, FoxO1, FoxO3, FoxO4 and FoxO6. While FoxO6 is rather specific for neurons, the other FoxOs are expressed in most tissues and are causally linked to cell survival, cellular proliferation and DNA damage repair response (*Monsalve* and *Olmos*, 2011). In humans, a 1.5 Kb region of chromosome 6, in or near intron 2, contains dozens of non-coding *foxO3* single nucleotide polymorphisms (SNPs), in high linkage disequilibrium (close association) (*Donlon* et al., 2012). These *foxO3* SNPs vary in frequency by ethnicity, but one or more SNPs are directly associated with life span in all populations. This finding has been observed in case-control studies of centenarians and shorter-lived controls as well as cohort studies of older individuals (*Broer* et al., 2015; *Santos-Lozano* et al., 2016). Furthermore, since the FoxO protein and its target sites are highly conserved, analyses of human, mouse, *C. elegans* and *Drosophila* homologs recently uncovered FoxO as being a central hub in regulating a network of ageing-related genes (*Webb* et al., 2016). Functional analyses in model organisms verified that the level of FoxO expression is indeed directly linked to life span without detectable costs for the individuals (*Kenyon* et al., 1993; *Hwangbo* et al., 2004; *Schaible* and *Sussman*, 2013). Several human tissues experience synchronized changes in gene expression during the ageing process which were shown to be a direct cause of age-related diseases (*Yang* et al., 2015). Furthermore, also the immune system is affected by the ageing process. "Immunosenescence" describes the deteriorating function of the immune system which results in increased susceptibility of elderly populations to infection, autoimmune diseases and cancer (*Pawalec*, 1999; *Castle*, 2000). The breakdown of mechanical barriers like the epithelia of lung, skin or intestinal tract leads to increased incidences of pathogenic invasion (*Gomez* et al., 2005). Combined with the declined functionality of immune cells and dysregulation of central components of innate immunity, elderly individuals observe a lower effectiveness in coping with the increased inflammation status, consequently resulting in higher morbidity and mortality (*Franceschi* et al., 2005; *Rosenstiel* et al., 2008). Although the understanding of FoxO signalling antagonizing ageing appears crucial to explain life span and health span in humans, the causative role of FoxO can only be addressed in model organisms.

MICROBES MATTER DURING THE AGEING PROCESS

All living beings are metaorganisms, associated with myriads of viruses, archaea and bacteria (*Zilber-Rosenberg* and *Rosenberg*, 2008; *Bosch* and *McFall-Ngai*, 2011). High throughput sequencing studies on humans showed that all epithelia are colonized with different, distinct microbial communities (*Eckberg* et al., 2005; *Grice* and *Segre*, 2011). Furthermore, many severe and chronic diseases, *e.g.* irritable bowel syndrome, inflammatory bowel disease, allergy or asthma, are associated with an altered bacterial

composition on the affected site (*Khosravi* and *Mazmanian*, 2013; *Fujimura* and *Lynch*, 2015). Studies in model organisms show that symbiotic bacteria can provide essential amino acids to the host (*Sandström* and *Moran*, 1999; *Shigenobu* et al., 2000), detoxify harmful substances (*Chaucheyras-Durand* et al., 2010) or degrade complex carbohydrates and thereby make them accessible to the host (*Warnecke* et al., 2007; *Keeney* and *Finlay*, 2011). Additionally, the composition of the gut microbiota regulates the nutrition uptake and is involved in weight control in mice and humans (*Ley* et al., 2005, 2006). Therefore, an organism's health status is reliant on an intact gut microflora (*Goodman* et al., 2009; *Blottière* et al., 2013; *Khosravi* and *Mazmanian*, 2013). Recent research also underlines the importance of bacterial colonization on host behaviour (*Vuong* et al., 2017) as well as fundamental developmental processes involving cell proliferation and immune system maturation (*Rakoff-Nahoum* et al., 2004; *Mazmanian* et al., 2005; *Bates* et al., 2006; *Cheesman* et al., 2011; *Hill* et al., 2016).

Interestingly, the human microbiome is not stable throughout life but experiences significant changes with age. First inoculation occurs during birth as new-borns are colonized with birth canal specific taxa (*Dominguez-Bello* et al., 2016). Disruption of this transmission via caesarean section or prenatal use of antibiotics may increase the risk of coeliac disease, asthma or obesity (*Couzin-Frankel* et al., 2010; *Decker* et al., 2010; *Ajslev* et al., 2011; *Metsälä* et al., 2015) and therefore have a huge impact on the individual's health. The intestinal microbiota resembles an adult stage already at the age of three (*Yatsunenko* et al., 2012), while the community of the skin microbes still undergoes drastic changes in puberty before it reaches a robust state (*Oh* et al., 2012). Over adulthood the bacterial communities are very stable but start to change again in elderly cohorts. The ageing process affects the structure of the human gut microbiota which results in a decrease in species diversity and a higher risk of pathogenic infections (*Keller* and *Surawicz*, 2014). In model organisms the structure of the bacterial community is discussed to have a direct influence on the ageing process as it can be protective against pathogens, shapes the nutrient landscape and affects the inflammation status (*Heintz* and *Mair*, 2014). In the short-lived African turquoise killifish the microbial community of young donors is able to extend life span in older individuals by preventing the decrease in microbial diversity associated with host ageing (*Smith* et al., 2017). Concordantly, latest research in *C. elegans* describes the positive impact of certain bacterial genotypes on host life span and health as non-essential bacterial compounds, *e.g.* colanic acid, are able to regulate mitochondrial dynamics and unfolded protein response (UPR^{mt}) (*Han* et al., 2017).

NON-SENESCENT MODEL ORGANISMS TO STUDY THE INTERACTION BETWEEN FOXO AND MICROBES

While most ageing research has been done in short-living models, some animals show no ageing phenotype and are considered as non-senescent. The freshwater polyp *Hydra* (Figure 1A) has an estimated life span that exceeds 1400 years (*Jones* et al., 2014). It belongs to the class of *Hydrozoa* within

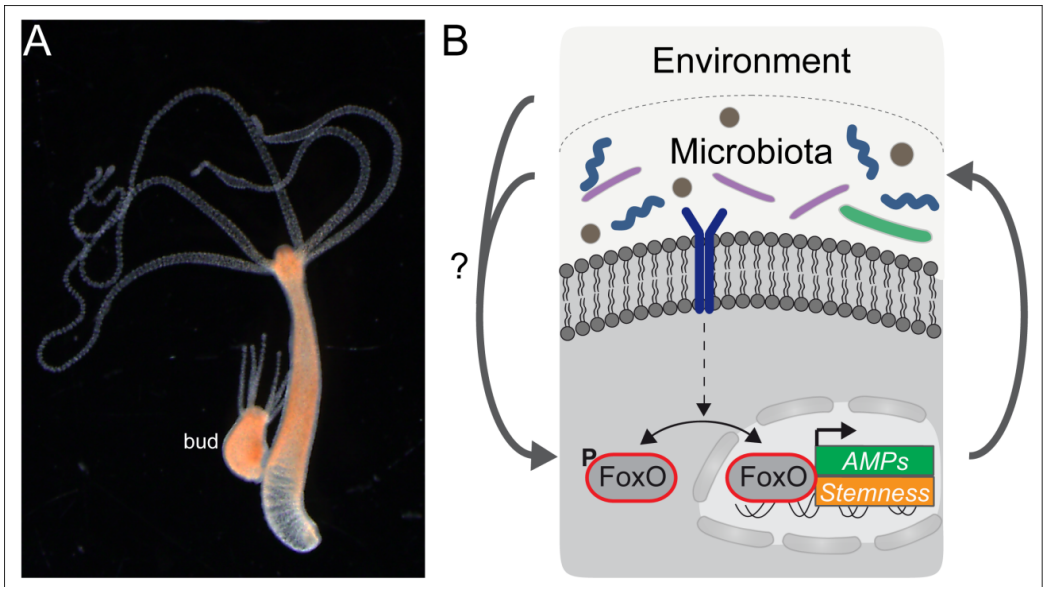

Figure 1: Conserved transcription factor FoxO controls non-senescence and innate immunity in Hydra. (A) Non-senescent Hydra. Continuous proliferation of the stem cell compartment provides the potential of an unlimited life span and clonal growth by asexual budding. (B) Model depicting the transcription factor FoxO as the key regulator of tissue maintenance and as link to the innate immune system. In response to environmental or bacterial signals FoxO can be shuttled between the transcriptionally inactive state in the cytoplasm and the active form in the nucleus. FoxO targets include genes of tissue maintenance and AMPs that directly loop back to the associated microbes. Therefore, FoxO is not only an ageing antagonist but could also be involved in maintenance of the metaorganism (from: *Mortzfeld* and *Bosch*, 2017).

the phylum of *Cnidaria*, the sister group of bilaterians, and therefore holds a basal position in the animal tree of life (*Martindale* et al., 2002). Due to three everlasting stem cell lineages that give rise to about 20 cell types (*Bosch*, 2009), the animals are able to continuously reproduce asexually via budding (Figure 1A). A single homolog of the longevity gene *foxO* was found to be expressed in all three stem cell lineages in *Hydra* (*Hemmrich* et al., 2012). While earlier *Hydra* studies associated the transcription factor with apoptosis (*Lasi* et al., 2010) and stress resistance (*Bridge* et al., 2010), functional analyses uncovered FoxO's role in both stem cell regulation and innate immunity (*Boehm* et al., 2012). *In situ* hybridizations of the cnidarians *Clytia* and *Hydra* revealed *foxO* expression in the area of cell proliferation (*Chevalier* et al., 2006; *Boehm* et al, 2012). Epithelial FoxO deficiency for the latter resulted in a significant increase of differentiated cell mass in relation to the stem cell compartment which resembles the ageing phenotype in senescent animals. Inversely, an overexpression in the interstitial stem cell lineage caused not only an increase in cell proliferation, but also expression of stem cell marker genes in terminally differentiated cells (*Boehm* et al., 2012). FoxO's capacity in other model organisms [*Drosophila* (*Hwangbo* et al., 2004) and *C. elegans* (*Kenyon* et al., 1993)] to delay the onset of age-related processes and to extend life span underlines its conserved potential in stem cell control and tissue maintenance, especially in the absence of classical stem cell

factors like Oct-3/4 or SOX2 (*Chapman* et al., 2010). Remarkably, the changes in tissue homeostasis were accompanied by altered expression of antimicrobial peptides (AMPs), effectors of the innate immune system.

Microbial colonization has been studied intensively in cnidarians as they have a huge impact on marine ecology but also offer a great opportunity to study bacteria-host interactions in organisms of lower complexity. Intracellular photosynthetic dinoflagellates (*e.g. Symbiodinium*) allow corals to populate nutrient-poor environments (*Muscatine* and *Porter*, 1977), however, recent research has shown that basal metazoans in addition to eukaryotic photobionts also harbour extracellular, species-specific bacterial communities (*Rohwer* et al., 2002; *Littman* et al., 2009; *Franzenburg* et al., 2013a). In the case of *Hydra* even closely related species are colonized by specific microbiomes that mirror the phylogenetic relatedness between their host species (phylosymbiosis; *Brooks* et al., 2016). The species-specific microbiomes in cnidarians are extremely stable and still resemble the ones of animals from the wild even after years of culturing under laboratory conditions involving artificial water and feeding (*Fraune* and *Bosch*, 2007; *Franzenburg* et al., 2013a; *Mortzfeld* et al., 2016). The acquisition of bacterial symbionts occurs during early development and establishes in a robust, reproducible pattern (*Franzenburg* et al., 2013b; *Mortzfeld* et al., 2016). Specific bacterial taxa may not only be acquired horizontally (*Apprill* et al., 2009; *Sharp* et al., 2010) but in some cases also via vertical transmission (parent to offspring) (*Fraune* et al., 2010; *Sharp* et al., 2012). Certain taxa of free-living bacteria can even be attracted by the host using chemotactic gradients (*Tout* et al., 2015). Underlining the importance of the microbiome, a study with germ-free animals showed that *Hydra* is protected from fungal infections by its bacterial colonizers. Interestingly, recolonization experiments proofed that a combination of the two most abundant bacteria of the naturally occurring microbiota [*Curvibacter sp.* 75% and *Duganella sp.* 10% (*Franzenburg* et al., 2013a)] was most protective, while monoassociations resulted in insufficient effects (*Fraune* et al., 2015).

AGEING ANTAGONIST FOXO CONTROLS BOTH STEM CELLS AND MICROBES

Besides its well-known conserved function as major tissue regulator and ageing antagonist, FoxO modulates the innate immune system in various model organisms including mouse (*Seiler* et al., 2013), *Drosophila* (*Becker* et al., 2010), *C. elegans* (*Libina* et al., 2003) and *Hydra* (*Boehm* et al., 2012). In mice FoxO signalling has been shown to reduce susceptibility to bacterial infections by reducing oxidative stress and induction of inflammatory cytokines (*Joseph* et al., 2016). Furthermore, FoxO transcription factors directly regulate TLR3-mediated innate immune responses as well as the expression of AMPs and thereby contribute to pathogen clearance in the respiratory tract (*Seiler* et al., 2013). Also in *Drosophila* AMPs are well-known effector molecules of the innate immune system and important regulators of the bacterial colonizers. Here, oral bacterial infection induces FoxO activity in the intestine, while impaired FoxO signalling decreases resistance to

intestinal infections. The inability to raise the expression level of AMPs leads to an elevated bacterial load and a decline in survival (*Fink* et al., 2015). In *Hydra*, the microbiome is selectively assembled by a species-specific combination of AMPs which are predominantly expressed in epithelial cells (*Fraune* et al., 2010; *Franzenburg* et al., 2013a). FoxO-deficient *Hydra* polyps show in addition to defects in stem cell maintenance a severe change of the immune status and drastically altered expression of AMPs (*Boehm* et al., 2012). Remarkably, loss of tissue homeostasis as well as AMP-deficiency compromise the ability to select for microbial communities resembling the polyps' native microbiota (*Fraune* et al., 2009; *Franzenburg* et al., 2013a). Interestingly, a recent study indicates that FoxO signalling may also be involved in the establishment of symbiosis in the early developmental phase of the sea anemone *Aiptasia* (*Wolfowicz* et al., 2016).

Taken together, the conserved transcription factor FoxO appears to combine two functions crucially involved in ageing and health in metazoans (Figure 1B): FoxO is responsible for stem cell regulation, including tissue maintenance and renewal, and controls the innate immune system. In response to environmental (including bacterial) signals, FoxO switches between a transcriptionally inactive state in the cytoplasm and an active form in the nucleus thereby serving as an intracellular control board for environmental signals. The capabilities of the FoxO transcription factor to extend life span and control effectors of the immune system demonstrate a strong and unique mechanism of cross-regulation of tissue homeostasis and innate immunity.

By exploring epithelial FoxO loss-of-function mutants, we recently made two important discoveries (*Mortzfeld* et al., 2018). First, deficiency in FoxO signalling leads to dysregulation of multiple AMP families. Most genes encoding epithelially expressed AMP families including Hydramacin, Kazal and Arminin respond with downregulation to FoxO-deficiency. Only one gene (contig 45266) was found to be upregulated in FoxO deficient animals, suggesting a mainly activating function of FoxO signalling on AMP expression and innate immunity. Second, FoxO loss-of-function polyps were more susceptible to colonization of foreign bacteria and impaired in selection for bacteria resembling the native microbiome. Therefore, FoxO-induced decrease in AMP expression is correlating with differences in microbial colonization and highlights the inhibitory action of AMPs against non-commensal bacteria. In a state of intact FoxO signalling secretion of numerous AMP families provides a highly selective milieu and shapes the microbial composition in a species-specific manner (Figure 1B). FoxO-deficiency reduces the expression of AMPs, which results in a decreased selection pressure on colonizing taxa and in establishment of higher abundances of foreign bacteria in the community. Consequently, especially during the process of colonization, reduced expression of FoxO compromises the resilience of the microbiome.

CONCLUSIONS

Stem cell maintenance and immunity are two of the prominent areas in ageing research. Observations in different organisms indicate that, in contrast

to the essentially static genome, the microbiome is rather dynamic throughout life history. Our observations in non-senescent *Hydra* add support to the view that (i) there is a need to consider the holobiotic nature of an organism when thinking about longevity, that (ii) the microbial environment matters in the context of senescence and contributes to complex processes such as ageing; and that (iii) the hub regulator FoxO presents a direct link between age-related processes and microbial colonization. In the newly discovered world of metaorganisms, stem cell proliferation and immunity are part of a global program, which seeks to fuse stem cell biology with ecological concepts and the rules governing the interactions between an organism and its microbial environment (*Mortzfeld* and *Bosch*, 2017; *Mortzfeld* et al., 2018).

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LITERATURE

Ajslev, T.A., Andersen, C.S., Gamborg, M., Sørensen, T.I., and Jess, T.: Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. Int. J. Obes. (Lond). 35, 522-529 (2011).

Apprill, A., Marlow, H.Q., Martindale, M.Q., and Rappé, M.S.: The onset of microbial associations in the coral Pocillopora meandrina. ISME J. 3, 685-699 (2009).

Bates, J.M., Mittge, E., Kuhlman, J., Baden, K.N., Cheesman, S.E., and Guillemin, K.: Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Dev. Biol. 297, 374-386 (2006).

Becker, T., Loch, G., Beyer, M., Zinke, I., Aschenbrenner, A.C., Carrera, P., Inhester, T., Schultze, J.L., and Hoch, M.: FOXO-dependent regulation of innate immune homeostasis. Nature 463, 369-373 (2010).

Bennet, A.M., Di Angelantonio, E., Ye, Z., Wensley, F., Dahlin, A., Ahlbom, A., Keavney, B., Collins, R., Wiman, B., de Faire, U., and Danesh, J.: Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA 298, 1300-1311 (2007).

Blottière, H.M., de Vos, W.M., Ehrlich, S.D., and Doré, J.: Human intestinal metagenomics: state of the art and future. Curr. Opin. Microbiol. 16, 234-239 (2013).

Boehm, A.M., Khalturin, K., Anton-Erxleben, F., Hemmrich, G., Klostermeier, U.C., Lopez-Quintero, J.A., Oberg, H.H., Puchert, M., Rosenstiel, P., Wittlieb, J., and Bosch, T.C.: FoxO is a critical regulator of stem cell maintenance in immortal Hydra. Proc. Natl. Acad. Sci. USA 109, 19697-19702 (2012).

Bosch, T.C.G.: Hydra and the evolution of stem cells. BioEssays 31, 478-486 (2009).

Bosch, T.C.G. and McFall-Ngai, M.J.: Metaorganisms as the new frontier. Zoology 114, 185-190 (2011).

Bourne, D.G., Morrow, K.M., and Webster, N.S.: Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. Annu. Rev. Microbiol.

70, 317-340 (2016).

Bridge, D., Theofiles, A.G., Holler, R.L., Marcinkevicius, E., Steele, R.E., and Martínez, D.E.: FoxO and stress responses in the cnidarian Hydra vulgaris. PLoS One 5, e11686 (2010).

Broer, L., Buchman, A.S., Deelen, J., Evans, D.S., Faul, J.D., Lunetta, K.L., Sebastiani, P., Smith, J.A., Smith, A.V., Tanaka, T., Yu, L., Arnold, A.M., Aspelund, T., Benjamin, E.J., De Jager, P.L., Eirkisdottir, G., Evans, D.A., Garcia, M.E., Hofman, A., Kaplan, R.C., Kardia, S.L., Kiel, D.P., Oostra, B.A., Orwoll, E.S., Parimi, N., Psaty, B.M., Rivadeneira, F., Rotter, J.I., Seshadri, S., Singleton, A., Tiemeier, H., Uitterlinden, A.G., Zhao, W., Bandinelli, S., Bennett, D.A., Ferrucci, L., Gudnason, V., Harris, T.B., Karasik, D., Launer, L.J., Perls, T.T., Slagboom, P.E., Tranah, G.J., Weir, D.R., Newman, A.B., van Duijn, C.M., and Murabito, J.M.: GWAS of Longevity in CHARGE Consortium Confirms APOE and FOXO3 Candidacy. J. Gerontol. A Biol. Sci. Med. Sci. 70, 110-118 (2015).

Brooks, A.W., Kohl, K.D., Brucker, R.M., van Opstal, E.J., and Bordenstein, S.R.: Phylosymbiosis: Relationships and Functional Effects of Microbial Communities across Host Evolutionary History. PLoS Biol 14, e2000225 (2016).

Castle, S.C.: Clinical relevance of age-related immune dysfunction. Clin. Infect. Dis. 31, 578-585 (2000).

Chapman, J.A., Kirkness, E.F., Simakov, O., Hampson, S.E., Mitros, T., Weinmaier, T., Rattei, T., Balasubramanian, P.G., Borman, J., Busam, D., Disbennett, K., Pfannkoch, C., Sumin, N., Sutton, G.G., Viswanathan, L.D., Walenz, B., Goodstein, D.M., Hellsten, U., Kawashima, T., Prochnik, S.E., Putnam, N.H., Shu, S., Blumberg, B., Dana, C.E., Gee, L., Kibler, D.F., Law, L., Lindgens, D., Martinez, D.E., Peng, J., Wigge, P.A., Bertulat, B., Guder, C., Nakamura, Y., Ozbek, S., Watanabe, H., Khalturin, K., Hemmrich, G., Franke, A., Augustin, R., Fraune, S., Hayakawa, E., Hayakawa, S., Hirose, M., Hwang, J.S., Ikeo, K., Nishimiya-Fujisawa, C., Ogura, A., Takahashi, T., Steinmetz, P.R., Zhang, X., Aufschnaiter, R., Eder, M.K., Gorny, A.K., Salvenmoser, W., Heimberg, A.M., Wheeler, B.M., Peterson, K.J., Böttger, A., Tischler, P., Wolf, A., Gojobori, T., Remington, K.A., Strausberg, R.L., Venter, J.C., Technau, U., Hobmayer, B., Bosch, T.C., Holstein, T.W., Fujisawa, T., Bode, H.R., David, C.N., Rokhsar, D.S., and Steele, R.E.: The dynamic genome of Hydra. Nature 464, 592-596 (2010).

Chaucheyras-Durand, F., Masséglia, S., Fonty, G., and Forano, E.: Influence of the composition of the cellulolytic flora on the development of hydrogenotrophic microorganisms, hydrogen utilization, and methane production in the rumens of gnotobiotically reared lambs. Appl. Environ. Microbiol. 76, 7931-7937 (2010).

Cheesman, S.E., Neal, J.T., Mittge, E., Seredick, B.M., and Guillemin, K.: Epithelial cell proliferation in the developing zebrafish intestine is regulated by the Wnt pathway and microbial signaling via Myd88. Proc. Natl. Acad. Sci. USA 108, 4570-4577 (2011).

Chevalier, S., Martin, A., Leclère, L., Amiel, A., and Houliston, E.: Polarised expression of FoxB and FoxQ2 genes during development of the hydrozoan Clytia hemisphaerica. Dev. Genes Evol. 216, 709-720 (2006).

Couzin-Frankel, J.: Bacteria and asthma: untangling the links. Science 330, 1168-1169 (2010).

Dato, S., Rose, G., Crocco, P., Monti, D., Garagnani, P., Franceschi, C., and Passarino, G.: The genetics of human longevity: an intricacy of genes, environment, culture and microbiome. Mech. Ageing Dev. 165, 147-155 (2017).

Decker, E., Engelmann, G., Findeisen, A., Gerner, P., Laass, M., Ney, D., Posovszky, C., Hoy, L., and Hornef, M.W.: Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. Pediatrics 125, e1433-40 (2010).

Dominguez-Bello, M.G., De Jesus-Laboy, K.M., Shen, N., Cox, L.M., Amir, A., Gonzalez, A., Bokulich, N.A., Song, S.J., Hoashi, M., Rivera-Vinas, J.I., Mendez, K., Knight, R., and Clemente, J.C.: Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nat. Med. 22, 250-253 (2016).

Donlon, T.A., Curb, J.D., He, Q., Grove, J.S., Masaki, K.H., Rodriguez, B., Elliott, A., Willcox, D.C., and Willcox, B.J.: FOXO3 gene variants and human aging: coding variants may not be key players. J. Gerontol. A Biol. Sci. Med. Sci. 67, 1132-1139 (2012).

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., and Relman, D.A.: Diversity of the Human Intestinal Microbial Flora. Science 308, 1635-1638 (2005).

Fink, C., Hoffmann, J., Knop, M., Li, Y., Isermann, K., and Roeder, T.: Intestinal FoxO signaling is required to survive oral infection in Drosophila. Mucosal Immunol. 9, 927-936 (2015).

Franceschi, C., Olivieri, F., Marchegiani, F., Cardelli, M., Cavallone, L., Capri, M., Salvioli, S., Valensin, S., De Benedictis, G., Di Iorio, A., Caruso, C., Paolisso, G., and Monti, D.: Genes involved in immune response/inflammation, IGF1/insulin pathway and response to oxidative stress play a major role in the genetics of human longevity: The lesson of centenarians. Mech. Ageing Dev. 126, 351-361 (2005).

Franzenburg, S., Walter, J., Künzel, S., Wang, J., Baines, J.F., Bosch, T.C.G., and Fraune, S.: Distinct antimicrobial peptide expression determines host species-specific bacterial associations. Proc. Natl. Acad. Sci. USA 110, E3730-8 (2013a).

Franzenburg, S., Fraune, S., Altrock, P.M., Künzel, S., Baines, J.F., Traulsen, A., and Bosch, T.C.G.: Bacterial colonization of Hydra hatchlings follows a robust temporal pattern. ISME J. 7, 781-790 (2013b).

Fraune, S. and Bosch, T.C.G.: Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. Proc. Natl. Acad. Sci. USA 104, 13146-13151 (2007).

Fraune, S., Abe, Y., and Bosch, T.C.G.: Disturbing epithelial homeostasis in the metazoan Hydra leads to drastic changes in associated microbiota. Environ. Microbiol. 11, 2361-2369 (2009).

Fraune, S., Augustin, R., Anton-Erxleben, F., Wittlieb, J., Gelhaus, C., Klimovich, V.B., Samoilovich, M.P., and Bosch, T.C.G.: In an early branching metazoan, bacterial colonization of the embryo is controlled by maternal antimicrobial peptides. Proc. Natl. Acad. Sci. USA 107, 18067-18072 (2010).

Fraune, S., Anton-Erxleben, F., Augustin, R., Franzenburg, S., Knop, M., Schröder, K., Willoweit-Ohl, D., and Bosch, T.C.G.: Bacteria-bacteria interactions within the microbiota of the ancestral metazoan Hydra contribute to fungal resistance. ISME J. 9, 1543-1556 (2015).

Fraune, S., Forêt, S., and Reitzel, A.M.: Using Nematostella vectensis to Study the Interactions between Genome, Epigenome, and Bacteria in a Changing Environment. Front. Mar. Sci. 3, 148 (2016).

Fujimura, K.E. and Lynch, S.V.: Microbiota in allergy and asthma and the emerging relationship with the gut microbiome. Cell Host Microbe 17, 592-602 (2015).

Gomez, C.R., Boehmer, E.D., and Kovacs, E.J.: The aging innate immune system. Curr. Opin. Immunol. 17, 457-462 (2005).

Goodman, A.L., McNulty, N.P., Zhao, Y., Leip, D., Mitra, R.D., Lozupone, C.A., Knight, R., and Gordon, J.I.: Identifying genetic determinants needed to establish a human gut symbiont in its habitat. Cell Host Microbe 6, 279-289 (2009).

Grice, E.A. and Segre, J.A.: The skin microbiome. Nat. Rev. Microbiol. 9, 244-253 (2011).

Han, B., Sivaramakrishnan, P., Lin, C.J., Neve, I.A.A., He, J., Tay, L.W.R., Sowa, J.N., Sizovs, A., Du, G., Wang, J., Herman, C., and Wang, M.C.: Microbial Genetic Composition Tunes Host Longevity. Cell 169, 1249-1262 (2017).

Heintz, C. and Mair, W.: You are what you

host: microbiome modulation of the aging process. Cell 156, 408-411 (2014).
Hemmrich, G., Khalturin, K., Boehm, A.M., Puchert, M., Anton-Erxleben, F., Wittlieb, J., Klostermeier, U.C., Rosenstiel, P., Oberg, H.H., Domazet-Lošo, T., Domazet-Loso, T., Sugimoto, T., Niwa, H., and Bosch, T.C.: Molecular signatures of the three stem cell lineages in hydra and the emergence of stem cell function at the base of multicellularity. Mol. Biol. Evol. 29, 3267-3280 (2012).
Hill, J.H., Franzosa, E.A., Huttenhower, C., and Guillemin, K.: A conserved bacterial protein induces pancreatic beta cell expansion during zebrafish development. Elife 5, e20145 (2016).
Hitt, R., Young-xu, Y., Silver, M., and Perls, T.: Centenarians : the older you get , the healthier you have been. Lancet 354, 2215 (1999).
Hwangbo, D.S., Gershman, B., Tu, M.P., Palmer, M., Tatar, M.: Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature 429, 562-566 (2004).
Jeck, W.R., Siebold, A.P., and Sharpless, N.E.: Review: a meta-analysis of GWAS and age-associated diseases. Aging Cell 11, 727-731 (2012).
Jones, O.R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C.G., Schaible, R., Casper, B.B., Dahlgren, J.P., Ehrlén, J., García, M.B., Menges, E.S., Quintana-Ascencio, P.F., Caswell, H., Baudisch, A., and Vaupel, J.W.: Diversity of ageing across the tree of life. Nature 505, 169-173 (2014).
Joseph, J., Ametepe, E.S., Haribabu, N., Agbayani, G., Krishnan, L., Blais, A., and Sad, S.: Inhibition of ROS and upregulation of inflammatory cytokines by FoxO3a promotes survival against Salmonella typhimurium. Nat. Commun. 7, 12748 (2016).
Keeney, K.M. and Finlay, B.B.: Enteric pathogen exploitation of the microbiota-generated nutrient environment of the gut. Curr. Opin. Microbiol. 14, 92-98 (2011).
Keller, J.M. and Surawicz, C.M.: Clostridium difficile infection in the elderly. Clin. Geriatr. Med. 30, 79-93 (2014).
Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtlang, R.: A C. elegans mutant that lives twice as long as wild type. Nature 366, 461-464 (1993).
Kenyon, C.J.: The genetics of ageing. Nature 464, 504-512 (2010).
Khosravi, A. and Mazmanian, S.K.: Disruption of the gut microbiome as a risk factor for microbial infections. Curr. Opin. Microbiol. 16, 221-227 (2013).
Lasi, M., David, C.N., and Böttger, A.: Apoptosis in pre-Bilaterians: Hydra as a model. Apoptosis 15, 269-278 (2010).
Ley, R.E., Backhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., and Gordon, J.I.: Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. USA 102, 11070-11075 (2005).
Ley, R., Turnbaugh, P., Klein, S., and Gordon, J.: Microbial ecology: human gut microbes associated with obesity. Nature 444, 1022-1023 (2006).
Libina, N., Berman, J.R., and Kenyon, C.: Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. Cell 115, 489-502 (2003).
Lindahl-Jacobsen, R., Tan, Q., Mengel-From, J., Christensen, K., Nebel, A., and Christiansen, L.: Effects of the APOE ε2 allele on mortality and cognitive function in the oldest old. J. Gerontol. A Biol. Sci. Med. Sci. 68, 389-394 (2013).
Littman, R.A., Willis, B.L., Pfeffer, C., and Bourne, D.G.: Diversities of coral-associated bacteria differ with location, but not species, for three acroporid corals on the Great Barrier Reef. FEMS Microbiol. Ecol. 68, 152-163 (2009).
López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G.: The hallmarks of aging. Cell 153, 1194-1217 (2013).
Martindale, M., Finnerty, J.R., and Henry, J.Q.: The Radiata and the evolutionary origin of the bilaterian body plan. Mol. Phylogenet. Evol. 24, 358-365 (2002).
Mazmanian, S.K., Cui, H.L., Tzianabos, A.O., and Kasper, D.L.: An immunomodulatory

molecule of symbiotic bacteria directs maturation of the host immune system. Cell 122, 107-118 (2005).

Metsälä, J., Lundqvist, A., Virta, L.J., Kaila, M., Gissler, M., and Virtanen, S.M.: Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. Clin. Exp. Allergy 45, 137-145 (2015).

Monsalve, M. and Olmos, Y.: The complex biology of FOXO. Curr. Drug Targets 12, 1322-1350 (2011).

Morris, B.J., Willcox, D.C., Donlon, T.A., and Willcox, B.J.: FOXO3: A Major Gene for Human Longevity--A Mini-Review. Gerontology 61, 515-525 (2015).

Mortzfeld, B.M., Urbanski, S., Reitzel, A.M., Künzel, S., Technau, U., and Fraune, S.: Response of bacterial colonization in Nematostella vectensis to development, environment and biogeography. Environ. Microbiol. 18, 1764-1781 (2016).

Mortzfeld, B. and Bosch, T.C.G.: Eco-Ageing: stem cells and microbes are controlled by ageing antagonist FoxO. Curr. Opin. Microbiol. 38, 181-187 (2017).

Mortzfeld, B.M., Taubenheim, J., Fraune, S., Klimovich, A.V., and Bosch, T.C.G.: Stem cell transcription factor FoxO controls microbiome resilience in Hydra. Front Microbiol., 9, 629 (2018).

Muscatine, L. and Porter, J.W.: Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27, 454-460 (1977).

Nebel, A., Kleindorp, R., Caliebe, A., Nothnagel, M., Blanché, H., Junge, O., Wittig, M., Ellinghaus, D., Flachsbart, F., Wichmann, H.E., et al.: A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals. Mech. Ageing Dev. 132, 324-330 (2011).

Oh, J., Conlan, S., Polley, E.C., Segre, J.A., and Kong, H.H.: Shifts in human skin and nares microbiota of healthy children and adults. Genome Med. 4, 77 (2012).

Pawelec, G.: Immunosenescence: impact in the young as well as the old? Mech. Ageing Dev. 108, 1-7 (1999).

Phillips, M.C.: Apolipoprotein e isoforms and lipoprotein metabolism. IUBMB Life 66, 616-623 (2014).

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., and Medzhitov, R.: Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 118, 229-241 (2004).

Rohwer, F., Seguritan, V., Azam, F., and Knowlton, N.: Diversity and distribution of coral-associated bacteria. Mar. Ecol. Prog. Ser. 243, 1-10 (2002).

Rosenstiel, P., Derer, S., Till, A., Häsler, R., Eberstein, H., Bewig, B., Nikolaus, S., Nebel, A., and Schreiber, S.: Systematic expression profiling of innate immune genes defines a complex pattern of immunosenescence in peripheral and intestinal leukocytes. Genes Immun. 9, 103-114 (2008).

Sandström, J. and Moran, N.: How nutritionally imbalanced is phloem sap for aphids? Entomol. Exp. Appl. 91, 203-210 (1999).

Santos-Lozano, A., Santamarina, A., Pareja-Galeano, H., Sanchis-Gomar, F., Fiuza-Luces, C., Cristi-Montero, C., Bernal-Pino, A., Lucia, A., and Garatachea, N.: The genetics of exceptional longevity: Insights from centenarians. Maturitas 90, 49-57 (2016).

Schächter, F., Faure-Delanef, L., Guénot, F., Rouger, H., Froguel, P., Lesueur-Ginot, L., and Cohen, D.: Genetic associations with human longevity at the APOE and ACE loci. Nat. Genet. 6, 29-32 (1994).

Schaible, R. and Sussman, M.: FOXO in aging: Did evolutionary diversification of FOXO function distract it from prolonging life? Bioessays 35, 1101-1110 (2013).

Schroeder, L.A. and Callaghan, W.M.: Thermal tolerance and acclimation of two species of Hydra. Limnol. Ocean. 26, 690-696 (1981).

Seiler, F., Hellberg, J., Lepper, P.M., Kamyschnikow, A., Herr, C., Bischoff, M., Langer, F., Schafers, H.J., Lammert, F., Menger, M.D., Bals, R., and Beisswenger, C.: FOXO transcription factors regulate innate immune mechanisms in respiratory epithelial cells. J. Immunol. 190, 1603-1613 (2013).

Sharp, K.H., Ritchie, K.B., Schupp, P.J., Ritson-Williams, R., and Paul, V.J.: Bacterial acquisition in juveniles of several broadcast spawning coral species. PLoS One 5, e10898 (2010).

Sharp, K.H., Distel, D., and Paul, V.J.: Diversity and dynamics of bacterial communities in early life stages of the Caribbean coral Porites astreoides. ISME J. 6, 790-801 (2012).

Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y., and Ishikawa, H.: Genome sequence of the endocellular bacterial symbiont of aphids Buchnera sp. APS. Nature 407, 81-86 (2000).

Smith, P., Willemsen, D., Popkes, M., Metge, F., Gandiwa, E., Reichard, M., and Valenzano, D.R.: Regulation of life span by the gut microbiota in the short-lived African turquoise killifish. Elife 6, e27014 (2017).

Tout, J., Jeffries, T.C., Petrou, K., Tyson, G.W., Webster, N.S., Garren, M., Stocker, R., Ralph, P.J., and Seymour, J.R.: Chemotaxis by natural populations of coral reef bacteria. ISME J. 9, 1764-1777 (2015).

Van Der Heide, L.P., Hoekman, M.F.M., and Smidt, M.P.: The ins and outs of FoxO shuttling: mechanisms of FoxO translocation and transcriptional regulation. Biochem. J. 380, 297-309 (2004).

Vuong, H.E., Yano, J.M., Fung, T.C., and Hsiao, E.Y.: The Microbiome and Host Behavior. Annu. Rev. Neurosci. 40, 21-49 (2017).

Warnecke, F., Luginbühl, P., Ivanova, N., Ghassemian, M., Richardson, T.H., Stege, J.T., Cayouette, M., McHardy, A.C., Djordjevic, G., Aboushadi, N., Sorek, R., Tringe, S.G., Podar, M., Martin, H.G., Kunin, V., Dalevi, D., Madejska, J., Kirton, E., Platt, D., Szeto, E., Salamov, A., Barry, K., Mikhailova, N., Kyrpides, N.C., Matson, E.G., Ottesen, E.A., Zhang, X., Hernández, M., Murillo, C., Acosta, L.G., Rigoutsos, I., Tamayo, G., Green, B.D., Chang, C., Rubin, E.M., Mathur, E.J., Robertson, D.E., Hugenholtz, P., and Leadbetter, J.R.: Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. Nature 450, 560-565 (2007).

Webb, A.E., Kundaje, A., and Brunet, A.: Characterization of the direct targets of FOXO transcription factors throughout evolution. Aging Cell 15, 673-685 (2016).

Willcox, B.J., Donlon, T.A., He, Q., Chen, R., Grove, J.S., Yano, K., Masaki, K.H., Willcox, D.C., Rodriguez, B., and Curb, J.D.: FOXO3A genotype is strongly associated with human longevity. Proc. Natl. Acad. Sci. USA 105, 13987-13992 (2008).

Williams, R.B.: Starlet sea anemone: Nematostella vectensis. In: The IUCN invertebrate red data book (Eds.: Collins, N.M., Hughes, S.A., Pyle, R.M., and Wells, S.). IUCN, Gland, Switzerland: 43-46 (1983).

Wolfowicz, I., Baumgarten, S., Voss, P.A., Hambleton, E.A., Voolstra, C.R., Hatta, M., and Guse, A.: Aiptasia sp. larvae as a model to reveal mechanisms of symbiont selection in cnidarians. Sci. Rep. 6, 32366 (2016).

Yang, J., Huang, T., Petralia, F., Long, Q., Zhang, B., Argmann, C., Zhao, Y., Mobbs, C.V., Schadt, E.E., Zhu, J., and Tu, Z.: Synchronized age-related gene expression changes across multiple tissues in human and the link to complex diseases. Sci. Rep. 5, 15145 (2015).

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., Heath, A.C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J.G., Lozupone, C.A., Lauber, C., Clemente, J.C., Knights, D., Knight, R., and Gordon, J.I.: Human gut microbiome viewed across age and geography. Nature 486, 222-227 (2012).

Ziegler, M., Seneca, F.O., Yum, L.K., Palumbi, S.R., and Voolstra, C.R.: Bacterial community dynamics are linked to patterns of coral heat tolerance. Nat. Commun. 8, 1-8 (2017).

Zilber-Rosenberg, I. and Rosenberg, E.: Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiol. Rev. 32, 723-735 (2008).