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Host and microbiota metabolic signals in aging and longevity

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Aging is an inevitable biochemical process that adversely affects personal health and poses ever-increasing challenges to society. Recent research has revealed the crucial role of metabolism in regulating aging and longevity. During diverse metabolic processes, the host organism and their symbiotic partners—the microbiota—produce thousands of chemical products (metabolites). Emerging studies have uncovered specific metabolites that act as signaling molecules to actively regulate longevity. Here we review the latest progress in understanding the molecular mechanisms by which metabolites from the host and/or microbiota promote longevity. We also highlight state-of-the-art technologies for discovering, profiling and imaging aging- and longevity-regulating metabolites and for deciphering the molecular basis of their actions. The broad application of these technologies in aging research, together with future advances, will foster the systematic discovery of aging- and longevity-regulating metabolites and their signaling pathways. These metabolite signals should provide promising targets for developing new interventions to promote longevity and healthy aging.

According to the World Health Organization, the proportion of people over 60 years of age in the total world population is expected to exceed 22% by the year 2050. While better healthcare and lifestyles promote longer lives, general aging is a primary risk factor for various human pathologies, such as cardiovascular disorders, cancer and neurodegenerative diseases. In 2018, aging-related pathologies all together accounted for more than 50% of all-cause mortality records in the United States. Meanwhile, centenarians, a group of human subpopulations, have exceptionally long lifespans with the absence of age-related pathologies before 80 years of age, providing good examples of longevity and healthy aging. In the past decades, multiple regulatory pathways of aging and longevity, which are well conserved across species, have been characterized using model organisms[1]. For example, reduced signaling through the insulin/insulin-like growth factor 1 (IGF-1) and mechanistic target of rapamycin (mTOR) pathways promotes longevity in organisms from roundworm (*Caenorhabditis elegans*) and fruit fly (*Drosophila melanogaster*) to mouse (*Mus musculus*)[1]. Interestingly, several longevity-related pathways have crucial roles in the control of metabolism. The dynamic processes of metabolism generate a variety of intermediates and products, collectively called metabolites. Metabolites are traditionally studied for their roles in appropriate biochemical pathways, with the aim of identifying biomarkers to diagnose and predict diseases. Excitingly, emerging studies have started to reveal the signaling role of certain metabolites as active drivers in diverse biological processes. In particular, systematic analyses of metabolomic changes associated with different longevity-regulatory pathways have identified a series of metabolites that are potential longevity regulators, including bioactive fatty acids and their derivatives, branched-chain amino acids (BCAAs), bile acids and polyamines.

Environmental factors affect aging. These include the trillions of microscopic residents coinhabiting the gastrointestinal tract—the gut microbiota, including bacteria, yeast and viruses. These microorganisms continuously exchange nutrients, genetic materials and metabolites with their host throughout the host's lifespan. Mutually, diverse internal and external factors, including host genetics, diet, antibiotics use and early microbial exposure, shape the composition of the gut microbiota, resulting in individual heterogeneity. Healthy gut microbiota is characterized by large bacterial taxonomic diversity[2]. While the major phylum of the microbiota remains stable across the human lifespan in healthy populations[3], the abundance and functionality of different microbial species fluctuate temporally during aging[2]. Such age-related imbalances in the microbiota lead to gut dysbiosis. This is a key attribute of intestinal barrier dysfunction accompanied by inflammation, which accelerates mortality in *Drosophila*[4] and is associated with aging-onset diseases in older humans[5]. On the other hand, specific microbiota signatures correlate with super-longevity in humans[6]. Fecal transplantation from young to old organisms delays age-related behavioral decline while prolonging lifespan in African turquoise killifish[7] and rescues systemic inflammation in mice[8]. Importantly, the taxonomic architecture of the gut microbiota affects not only intestinal permeability and inflammation but also microbial metabolism, as suggested by metagenomic and metabolomic analyses[9,10]. In the intestinal mucus layer, commensal bacteria generate numbers of microbial metabolites via bacterial de novo metabolism or by modifying host-derived molecules. Through active secretion and/or bacterial death and lysis, the host's gut is exposed to these metabolites, which mediate local intestinal homeostasis and also signaling from the gut to distant organs. Some of these bacterial metabolites regulate host longevity, such as short-chain fatty acids (SCFAs), exopolysaccharides, bile acids and polyamines.

Thus far, technological advancements in MS-based metabolomic analysis have expanded the pool of identified biologically active metabolites. Recent developments in chemical proteomics have pushed the limits of detection to allow systemic profiling of the protein partners of these metabolites and delineation of their regulatory pathways. Meanwhile, genetically encoded biosensors and chemical imaging techniques, such as stimulated Raman

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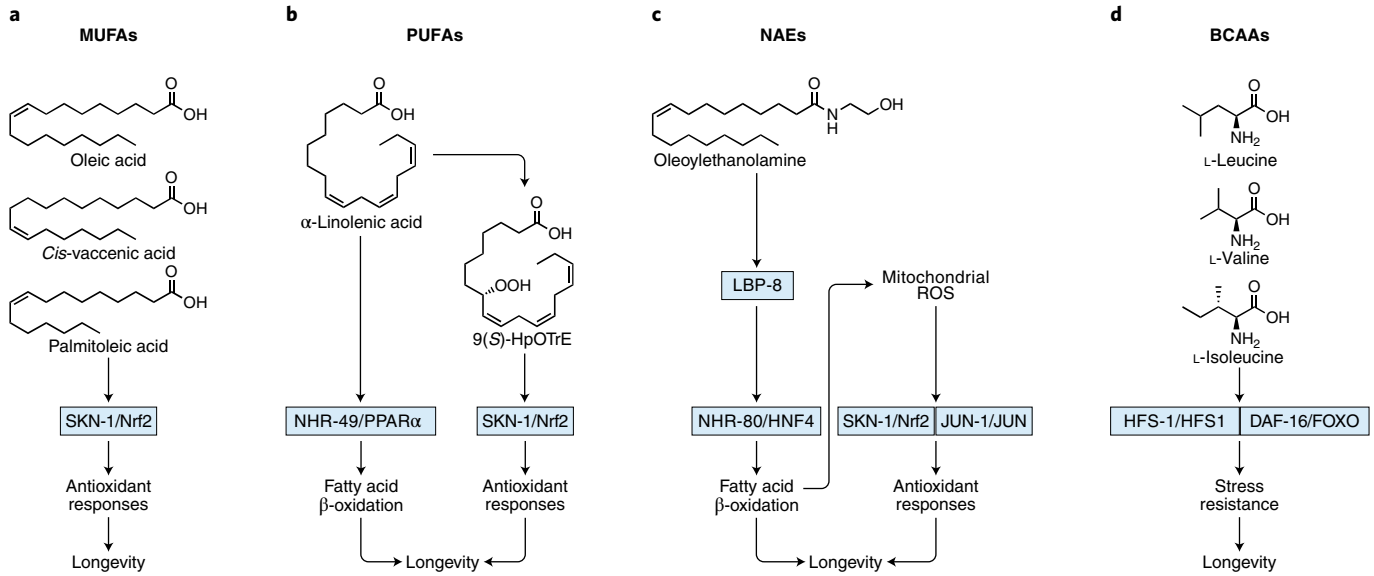

Fig. 1 | Chemical structures and mechanisms of aging-regulating host metabolites. a–d, MUFAs (**a**), PUFAs (**b**), NAEs (**c**) and BCAAs (**d**) are examples of host metabolites that modulate longevity through well-conserved aging-regulating transcription factors, including SKN-1/Nrf2, NHR-49/PPARα, JUN-1/JUN, HSF-1/HSF1 and DAF-16/FOXO. These representative aging-regulating pathways have been well studied in *C. elegans*. ROS, reactive oxygen species.

scattering (SRS) microscopy, make it possible to visualize metabolites and to track their spatiotemporal dynamics in vivo. In this Review, we present examples of host and microbiota metabolites with signaling roles in regulating aging and longevity. We also discuss methods that are driving new advances in studying metabolites and their biomedical impact, as well as the future potential of these methods in aging research.

Host metabolites regulate longevity

Aging has a profound impact on metabolism, leading to substantial changes in metabolite profiles. While the majority of age-associated metabolites are the results of aging, a few of them are potent longevity regulators. Supplementation with these metabolites not only prolongs lifespan but also provides protection from age-related pathologies. In this section, we use monounsatuated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), *N*-acylethanolamines (NAEs) and BCAAs as examples to discuss possible mechanisms by which signaling metabolites from the host contribute to longevity regulation (Fig. 1).

MUFAs and PUFAs. Fatty acids are lipophilic metabolites that consist of a saturated or unsaturated aliphatic chain with a carboxyl group at the end. MUFAs carry a single double bond on the acyl chain (Fig. 1a), whereas PUFAs contain multiple double bonds, usually with a single methylene spacer between two adjacent double bonds (Fig. 1b). Intracellular MUFAs and PUFAs exist as free fatty acids or as moieties on complex lipid species, such as glycerophospholipids, sphingolipids and triglycerides. PUFAs are more susceptible to peroxidation than MUFAs or saturated fatty acids[11]. Oxidation on PUFAs in membrane phospholipids generates carbon radicals that can react with dissolved oxygen molecules in the membrane to form highly reactive peroxyl radicals, leading to a chain reaction on nearby PUFAs and damaging membrane proteins[11]. The ratio of MUFAs to PUFAs has been linked to aging and longevity in different species. In *C. elegans*, a variety of mutants have been identified for their longevity-promoting effects[12]. Lipid profiling of some of these long-lived mutants has revealed that the accumulation of MUFA-containing lipids positively correlates with mean lifespan extension, with the longest-living mutant having the highest percentage of MUFAs in total lipid composition, while PUFAs are negatively correlated with longevity[12]. Meanwhile, in a familial lipidomic study of human longevity, the MUFA/PUFA ratio was higher in nonagenarian female offspring than in controls (spouses of the offspring)[13]. In line with these association studies, direct supplementation with MUFAs, including oleic acid (OA; C18:1n-9), palmitoleic acid (C16:1n-7) and *cis*-vaccenic acid (C18:1n-7), is sufficient to extend *C. elegans* lifespan[14]. While the mechanisms by which MUFAs extend lifespan need comprehensive investigation, OA was shown to associate with the epigenetic regulation of longevity[14] and to increase nuclear translocation of the SKN-1/Nrf2 (worm/mammalian homolog) transcription factor[15]. SKN-1/Nrf2 is a key modulator of the oxidative stress response and longevity in worms, fruit flies and mice[16], and the pro-longevity effect of OA in *C. elegans* is associated with reduced proteome-wide protein oxidation and improved protein homeostasis[17].

However, despite the tendency of an overall low PUFA/MUFA ratio to associate with longevity, certain PUFAs have been studied for their longevity-promoting effects, including arachidonic acid (AA), di-homo-γ-linoleic acid (DGLA) and α-linolenic acid (ALA). AA and DGLA are ω-6 PUFAs whose levels are elevated in fasted *C. elegans*, and supplementation with them individually activates autophagy pathways in mouse embryonic fibroblasts, as well as in well-fed worms, to prolong lifespan[18]. Supplementation with ALA, an ω-3 PUFA, also extends lifespan in *C. elegans*, which requires the nuclear hormone receptor NHR-49/PPARα and the SKN-1/Nrf2 transcription factor[19]. Interestingly, the peroxidation product from ALA, 9(*S*)-HpOTrE, but not ALA, activates SKN-1/Nrf2 and confers a synergetic effect with ALA on worm lifespan extension[19]. Thus, although PUFA peroxidation causes oxidative damage to the cellular membrane, oxidation products generated from this process may serve as signaling molecules to activate specific longevity-promoting pathways. Whether peroxidation products from other PUFAs exert similar signaling effects remains unclear. At the same time, both MUFAs and PUFAs may directly act as signaling molecules to regulate longevity, given their activities in binding to G-protein-coupled receptors (GPCRs), nuclear hormone receptors and fatty acid-binding proteins[15,20,21].

NAEs. NAEs are fatty acid amide derivatives (Fig. 1c) modified with ethanolamine, and the fatty acid chain can be saturated, monounsaturated or polyunsaturated[22]. In mammals, NAEs are signaling molecules that regulate a variety of physiological activities, such as energy metabolism, anxiety and inflammatory responses[22]. In *C. elegans*, two NAEs have been linked to longevity regulation: eicosapentaenoylethanolamide (EPEA) and oleoylethanolamine (OEA). EPEA is highly abundant in wild-type worms, but its levels are decreased following dietary restriction or inactivation of mTOR signaling, two conserved longevity mechanisms across various organisms[1]. EPEA supplementation suppresses the lifespan extension under these two conditions[23], supporting the idea that EPEA has a regulatory effect on aging. Thus far, how EPEA negatively regulates longevity remains unclear. OEA reduces food intake and body weight by activating the nuclear hormone receptor PPARα and lowers hepatic lipid accumulation in obese rodents[24]. In *C. elegans*, OEA is induced in a long-lived transgenic strain that constitutively expresses LIPL-4, a lysosomal acid lipase, and supplementation with an OEA analog is sufficient to promote longevity in wild-type worms[25]. Genetic and biochemical evidence has shown that OEA-associated longevity requires direct binding to the fatty acid-binding protein LBP-8 and the nuclear hormone receptor NHR-80/HNF4 (ref. [25]) (Fig. 1c). NHR-80/HNF4 interacts with NHR-49/PPARα to transcriptionally upregulate genes involved in mitochondrial β-oxidation, leading to increased lipid catabolism[25,26]. The metabolic changes in mitochondria further induce mitochondrial reactive oxygen species, which activate redox-sensitive transcription factors, including JUN-1/JUN and SKN-1/Nrf2, to induce antioxidant responses[25,26]. Together, the catabolic switch toward enhanced lipolysis and redox homeostasis contributes to increased longevity. Thus, different NAEs exert distinct effects on lifespan, likely through specific signaling mechanisms. Considering the high conservation of NAEs and their related regulatory components across species, it would be interesting to see further investigation of their effects on aging and longevity in mammals.

BCAAs. Amino acids are the building blocks of proteins and are also required for the biosynthesis of hormones and neurotransmitters. Of the 20 proteinogenic amino acids, 9 are essential to metazoans, which lack their biosynthesis and depend on ingestion from external sources. Three BCAAs—valine, leucine and isoleucine—are essential amino acids that contribute to metabolic homeostasis as both nutritional and signaling molecules (Fig. 1d)[27]. In the past decades, emerging studies in diverse organisms have revealed the crucial role of BCAAs in regulating aging and longevity. In yeast, supplementation with BCAAs extends chronological lifespan, which is mediated by a non-nutritional regulatory mechanism involving reduced expression of GCN4, a key transcriptional regulator of global amino acid homeostasis[28]. Furthermore, the gene encoding the BCAA transferase 1 (BCAT-1) enzyme, which localizes to mitochondria and catalyzes the first step of BCAA degradation, was identified as one of the 29 genes showing a significant change in expression during aging across three species: *C. elegans*, zebrafish and mice[29]. In *C. elegans*, *bcat-1* inactivation increases BCAA levels and extends lifespan, as does BCAA supplementation[29]. This lifespan extension is dependent on two transcription factors, HSF-1/HSF1 and DAF-16/FOXO, and also requires DAF-7/TGF-β signaling in ASI neurons, the *C. elegans* equivalent of the mammalian hypothalamus[29]. The same study further showed that *bcat-1* inactivation interacts with neuronal mTOR signaling to prolong lifespan[29]. In line with this, leucine is a well-known activator of mTOR, and hypothalamic leucine induces mTOR signaling to reduce food intake and body weight in mice[30]. However, mTOR activation per se does not promote longevity. Instead, both genetic and pharmaceutical suppression of mTOR signaling extend lifespan in yeast, *C. elegans*, *Drosophila* and mice[1].

On the other hand, elevated serum levels of BCAAs have been associated with insulin resistance in rodents and humans, although it remains unclear whether BCAAs are a causative driver or a biomarker in these cases[31]. In *C. elegans*, a similar elevation in BCAA levels has been detected in the long-lived *daf-2* mutant[32]. *daf-2* encodes the worm homolog of the insulin/IGF-1 receptor, and reduction of insulin/IGF-1 signaling with subsequent activation of the DAF-16/FOXO transcription factor is a well-conserved pro-longevity mechanism from *C. elegans* to humans[1]. In line with the link between BCAAs and insulin signaling, the lifespan extension conferred by *bcat-1* inactivation requires the DAF-16/FOXO transcription factor[29]. Moreover, supplementation with a BCAA-enriched mixture through drinking water in mice starting at 9 months of age extends lifespan, reduces mitochondrial oxidative stress in muscles and improves muscular endurance and coordination in middle-aged mice, without affecting insulin/IGF-1 levels, food intake or body weight[33]. In contrast, another study showed that increasing the proportion of BCAAs in dietary proteins shortened lifespan, resulted in increased body weight and caused obesity when the BCAA-enriched diet was fed to mice from 4 months of age[34]. These detrimental effects are not directly linked to additional BCAAs but are due to hyperphagia caused by amino acid imbalance, in particular, tryptophan-mediated serotonin depletion[34]. A more recent study showed that lifelong BCAA restriction extends the lifespan of wild-type male mice by inhibiting mTOR, while no lifespan extension is detected in females or if BCAA restriction starts in middle age (at 16 months)[35]. Therefore, the longevity regulation by BCAAs in mammals may be sensitive to variation in supplementation procedures and is complex, given their multifaceted effects on metabolism through interaction with both insulin and mTOR signaling.

Microbiota metabolites regulate host aging and longevity

In addition to host metabolism, the gut microbial community carries out a variety of metabolic activities that influence the local intestinal environment and impact distal organs systemically through circulation. Microbial metabolites derived from the gut microbiota have crucial roles in maintaining the host's metabolic fitness, endocrine homeostasis and brain health[36] and have been implicated in the regulation of longevity and age-related diseases. These metabolites can be unique to bacterial metabolism, shared by host and bacterial metabolism, or modified from host-derived molecules. In this section, we discuss examples in each of these three categories, including SCFAs and exopolysaccharides (unique to bacteria), bile acids (modified by bacteria) and polyamines (shared by the host and bacteria), for their longevity-regulatory mechanisms in different host models (Fig. 2).

SCFAs. SCFAs are fatty acids containing six or fewer carbons, including acetate, butyrate and propionate. SCFAs are predominantly produced through microbial fermentation in the colon from indigestible polysaccharides, such as fiber and resistant starch (Fig. 2a). Colonocytes absorb and use SCFAs as energy substrates and transport excess SCFAs into the circulation to reach other tissues. SCFAs are beneficial to intestinal homeostasis, systemic metabolism and brain function[37] and can bind to GPCRs and activate downstream signaling cascades[38]. The levels of microbial SCFAs decrease with increasing age[39,40]. The proportion of *Firmicutes*, a family of butyrate-producing bacteria, is significantly lower in older individuals than in young adults[41], while SCFAs remain abundant in centenarians[40]. Importantly, supplementation with SCFAs and bacteria producing them protects the host against age-related pathologies. For example, supplementation with butyrate-producing bacteria increases serum butyrate levels, reduces inflammation and restores insulin sensitivity in aged mice and macaques[42]. Through the gut–brain axis, butyrate supplementation inhibits neuronal amyloid

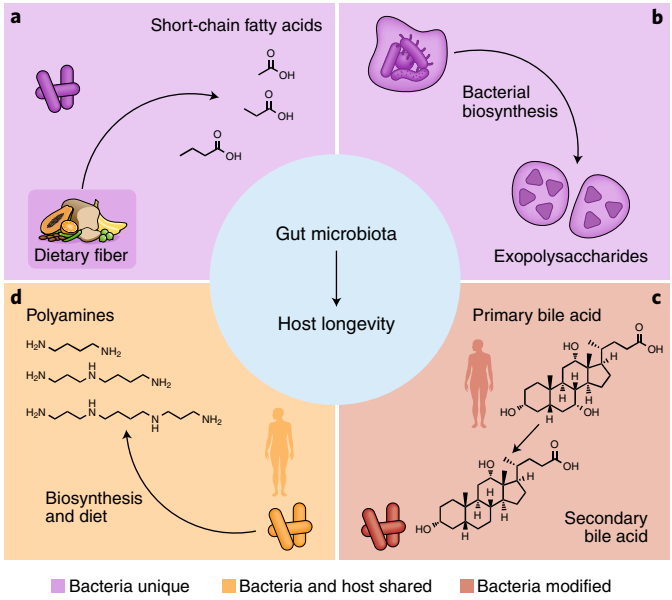

Fig. 2 | Microbial metabolites in aging regulation. a,b, SCFAs (**a**) and exopolysaccharides (**b**) are microbial metabolites derived from the microbiota. **c**, Cholic acid, representing primary bile acids from the host, and deoxycholic acid, representing secondary bile acids, indicate a pathway by which microbial metabolites are modified from host-derived compounds. **d**, Polyamines are metabolites produced by both the microbiota and the host.

formation and prevents memory impairment in Alzheimer's disease mouse models[43,44]. Moreover, SCFAs directly affect host epigenetic states, a hallmark of aging[1]. Histone deacetylation is a key attribute of aging-related disruptions in transcriptional responses[45]. As potent inhibitors of histone deacetylases, butyrate and propionate promote epigenetic changes on histones in the colon, liver and white adipose tissue[46,47] (Fig. 3a). In line with this, dietary supplementation with SCFAs extends lifespan in *C. elegans* and *Drosophila* by inhibiting histone deacetylation[48]. Therefore, SCFAs are promising candidates for promoting longevity and healthy aging. In addition to dietary intervention, it will be interesting to directly assess the lifespan-extending effects of bacteria-derived SCFAs in future probiotics studies.

Exopolysaccharides. Bacteria-derived carbohydrates have been intensively studied in the form of bacterial polysaccharides. These are complex carbohydrate polymers that can be defined as capsular polysaccharides, exopolysaccharides, lipopolysaccharides, teichoic acids and peptidoglycans. Many of these bacterial polysaccharides attach to the outer surface of bacteria and constitute a shield of protection against toxins in the environment[49]. Exopolysaccharides, on the other hand, are secreted into the environment and influence interactions between different species of bacteria, bacteria and the host, and bacteria and the environment[50] (Fig. 2b). Human intestinal *Bifidobacterium* isolates produce certain types of exopolysaccharides that are used as fermentation substrates by other bacterial species to promote SCFA production[40]. In zebrafish, direct supplementation with exopolysaccharides or an exopolysaccharide-producing probiotic *Lactobacillus* strain alters the composition of the gut microbiota, which is associated with health benefits[51]. Moreover, specific exopolysaccharides have a direct role in promoting host longevity. For example, colanic acid, an exopolysaccharide secreted by *Escherichia coli*, was identified for its pro-longevity effect through a genomic screen for microbial regulators of host aging[52]. In this screen, individual deletion of certain bacterial genes, including *lon* and *hns*, was found to promote healthy aging in the host *C. elegans* and induce the production of colanic acid[52]. Supplementation with colanic acid led to lifespan extension through regulation of mitochondrial dynamics and protected worms against age-associated amyloid-β accumulation and germline tumor progression[52] (Fig. 3b). The biosynthesis and secretion of colanic acid are controlled by the *cps* operon, which consists of 19 genes encoding enzymes[53]. Optogenetic induction of the *cps* operon in live bacteria residing in the lumen of the host gut is sufficient to modulate mitochondrial dynamics locally and to prolong lifespan[54]. Thus, in addition to modifying taxonomic composition, direct manipulation of bacterial gene expression and metabolite production in the microbiota offers another effective intervention to promote host longevity.

Bile acids. Bile acids are cholesterol derivatives that are produced by the liver in their primary forms. A small portion of bile acids reach the intestine and colon, where they are modified to secondary bile acids by gut bacteria through deconjugation and dehydroxylation (Fig. 2c). Regulation of longevity by bile acids was first observed in Little mice, which are deficient for growth hormone and live 20% longer than their wild-type littermates[55]. The increased levels of bile acids in these long-lived mice upregulate xenobiotic detoxification genes through the farnesoid X receptor[56]. Increased total bile acid levels have also been observed under methionine restriction, a well-conserved pro-longevity intervention[57,58]. High concentrations of taurocholate, a conjugate of cholic acid and taurine, strongly correlate with human longevity[59]. Directly supporting the pro-longevity effect of bile acids, supplementation with cholic acid, a primary bile acid, extends both healthspan and lifespan in progeroid mice[58], while supplementation with lithocholic acid, a secondary bile acid, prolongs the lifespan of both yeast[60] and fruit flies[61]. Moreover, systematic profiling and comparison of bile acids in young and old mice revealed an age-associated decrease in the ratio of secondary to primary bile acids, related to changes in the composition of the gut microbiota in old mice, that could be rescued by co-housing old mice with young individuals[62]. In humans, changes in bacteria-derived secondary bile acids have been associated with obesity, metabolic disorders, cardiovascular disease and other age-related complications[63]. Moreover, several types of lithocholic acid have been found at elevated levels in centenarians with distinct gut microbiota and exhibit potent antimicrobial activities in pathobiont infection[64]. Taking these findings

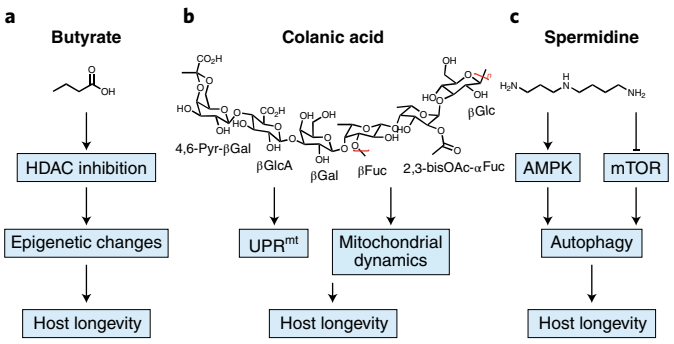

Fig. 3 | Mechanisms by which microbial metabolites regulate host longevity. a, Butyrate, an example of an SCFA, inhibits histone deacetylation to promote longevity in *C. elegans* and *Drosophila*. HDAC, histone deacetylase. **b**, Colanic acid, one type of exopolysaccharide secreted by *E. coli*, extends the lifespan of host *C. elegans* by modulating the mitochondrial unfolded protein response (UPR^{mt}) and mitochondrial dynamics. **c**, Spermidine, an example of a polyamine, promotes host longevity by inducing autophagy, which is mediated by AMPK activation and mTOR inhibition.

together, from yeast to humans, bile acids appear to be promising contributors to longevity, a role that is tightly intertwined with the metabolic function of the gut microbiota and worth further exploration at the mechanistic level.

Polyamines. Polyamines, including spermidine, spermine and putrescine, are polycationic molecules that are essential to cell growth, proliferation and survival[65]. Both eukaryotes and prokaryotes, including the gut microbiota, synthesize and catabolize polyamines[66] (Fig. 2d). In addition to endogenous biosynthesis, dietary polyamines are absorbed through the small intestine and the microbiota releases polyamines to the host in the lower intestinal tract[66]. During aging, polyamine levels decrease in different animal tissues, as well as in human serum[67], with a concomitant reduction in the enzymatic activity of ornithine decarboxylase-1, the rate-limiting enzyme for de novo polyamine synthesis[68]. Interestingly, centenarians exhibit increased levels of polyamines in comparison to control individuals[69]. Dietary supplementation with polyamines prolongs lifespan in a wide variety of organisms from yeast to mice[70,71]. A polyamine precursor, agmatine, effectively prolongs the lifespan of *C. elegans* when given by direct supplementation or agminate-producing *E. coli*[72,73]. Spermidine supplementation promotes healthy aging by protecting animals against age-related pathologies, such as memory impairment[74]. Supplementation with the probiotic strain *Bifidobacterium animalis* subsp. *lactis* LKM512 increases polyamine levels in the intestinal lumen and prolongs lifespan in mice, as well as ameliorating age-related colonic inflammation[75]. Probiotic supplementation with LKM512 in combination with arginine, another polyamine precursor, further increases colonic putrescine and serum spermidine and spermine levels, which in turn promotes longevity and protects aged mice from inflammation and memory impairment[76]. Moreover, in human cohort studies, nutrition rich in spermidine is associated with low mortality and increased survival[77]. To exert these beneficial effects in multiple systems, polyamines are known to induce autophagy[70], a key mediator of longevity[1]. This polyamine-induced autophagy may be mediated by several different but interconnected mechanisms, including decreased acetylation, inhibition of mTOR and activation of AMP-activated protein kinase (AMPK)[78] (Fig. 3c). Overall, polyamines are potent candidates to promote longevity and healthy aging through dietary intervention and microbiota manipulation.

Advances in methods for metabolite research in aging

Metabolites are highly dynamic in response to cellular actions and environmental inputs, naturally heterogeneous with immense chemical diversity and difficult to amplify for detection. These unique properties make the analysis of metabolites more challenging than that of DNA, RNA or protein. Recent advances in analytical and imaging methods have made it possible to identify bioactive metabolites, visualize their spatiotemporal dynamics and study their regulatory mechanisms. Some of these methods have been applied to the study of metabolite signals in aging and longevity regulation. In this section, we provide an overview of four methods for studying aging-related metabolites, including comparative metabolomics, chemical proteomics, biosensors and SRS microscopy. We also provide perspectives for future application of these technologies in aging research.

Comparative metabolomics to discover longevity signals. Comparative MS-based metabolomics (Fig. 4) identifies aging-related metabolites by comparing metabolomic data from different biological sources, such as wild-type organisms versus long-lived mutants, offspring from long-lived individuals versus the offspring's partners, and longitudinal samples in cross-sectional studies from different age groups. As previously mentioned, a variety of longevity-promoting metabolites were first identified through comparative MS-based metabolomics in long-lived animal models

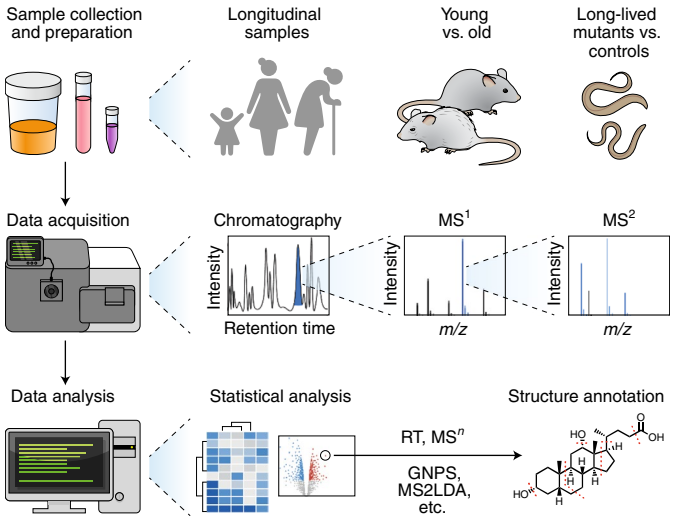

Fig. 4 | Comparative metabolomics in aging research. Metabolites from different age groups or long-lived mutants versus controls can be profiled with high-performance liquid chromatography coupled to tandem MS. Metabolic compounds with significant differences in their levels between groups are annotated for their chemical structures. Global metabolite data sharing systems (for example, GNPS) and the application of AI platforms (for example, MS2LDA) further improve chemical annotation. RT, retention time; *m/z*, mass-to-charge ratio.

and then confirmed to have benefit through direct supplementation[14,25,29]. Metabolomics can also profile microbial metabolites[9,66,79]. For example, a metabolomic analysis in a population-based twin study revealed that over 1,000 fecal metabolites are associated with age. Comprehensive comparison of the metabolic profiles from intestinal luminal biofluid highlighted an association between healthy gut microbiota and the production of specific polyamines, as well as functional peptides and prostaglandin E_2 (ref. [66]). Precise measurement of SCFAs through targeted metabolomics has shown an age-associated reduction that links metabolism in the microbiota to host cognitive decline[80].

In recent years, with improvements in instrumentation and sample preparation, MS-based metabolomics is detecting increasing numbers of metabolites with high accuracy. Notably, the rapid advances in single-cell metabolomics in the past five years have made it possible to detect a few hundred metabolites from a single cell and to quantitatively compare them under different physiological conditions[81]. While structural annotation of unknown metabolites is still challenging, recent applications of artificial intelligence (AI) in metabolomic data analysis and the community sharing of metabolite spectrum resources, such as on the Global Natural Products Social Molecular Networking (GNPS) platform[82], provide promising ways to accelerate structural elucidation of metabolites[83,84]. These advances will facilitate more applications of MS-based metabolomics in aging research to discover not only new biomarkers but also novel active regulators of healthy aging and longevity.

Chemical proteomics to profile metabolite partners. Metabolites often exert different regulatory effects by interacting with distinct proteins. While some protein regulators and their related pathways have been elucidated using conventional genetic screens, many others remain elusive. Chemical proteomics provides an unbiased and systematic approach to identify direct protein binding targets of metabolites.

Chemical proteomics with metabolite-specific diazirine alkyne (DA) probes can directly pull down metabolite-binding targets from living cells (Fig. 5a). For example, bile acids, as aging signaling

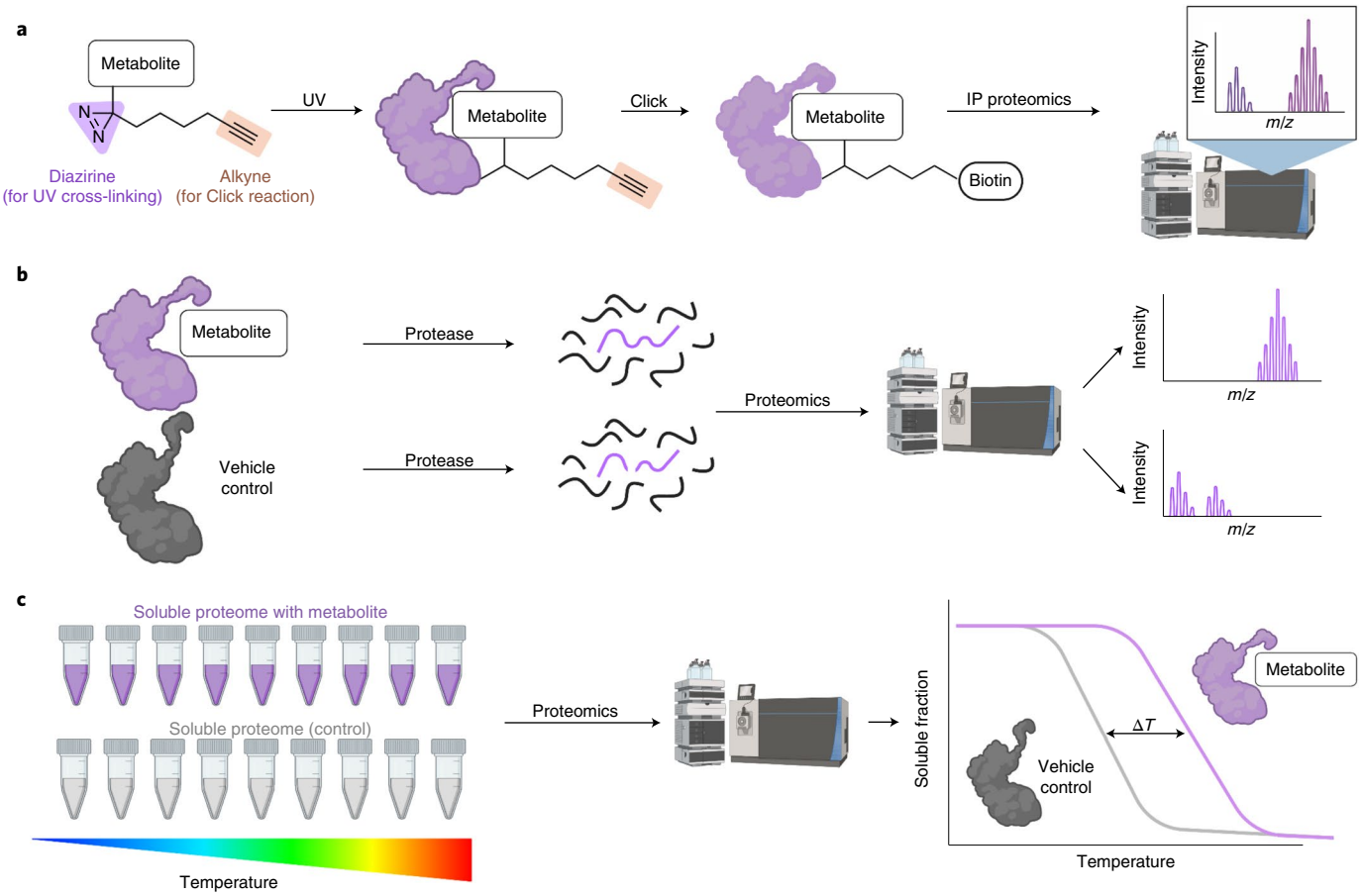

Fig. 5 | Chemical proteomics for identifying metabolite-binding targets. a, DA probe-based proteomics uses DA probe-modified metabolites. The diazirine moiety of the DA probe can be covalently cross-linked to proteins binding metabolites by UV light, and the alkyne moiety can be tagged to biotin through a Click reaction. The probe-linked proteins are then enriched with anti-biotin beads through immunoprecipitation (IP) and profiled using proteomics. **b**, LiP–MS relies on protein conformational changes following metabolite binding and detects peptide shifts after protease treatment. **c**, TPP detects thermal stability changes in proteins. For proteins binding metabolites, conformational changes often increase the denaturing temperature and result in a shift in the association between the soluble fraction and temperature.

molecules, have long been recognized to improve insulin sensitivity in diabetic mice by reducing endoplasmic reticulum (ER) stress[85] and to protect from Alzheimer's disease in mouse models[86]; however, the underlying molecular mechanisms remain unclear. A DA probe specified for cholic acid, one of the primary bile acids, has been developed and applied to profile the binding proteome of cholic acid, leading to the discovery of specific amyloid-β-processing enzymes and ER stress regulatory proteins that directly bind to bile acids[87]. This study provided new opportunities to understand the molecular mechanisms by which bile acids improve glucose homeostasis and insulin sensitivity in diabetic mice and attenuate amyloid-β accumulation in Alzheimer's disease models. Given the close link between healthy aging and these metabolic and neuronal protective effects, these new targets also shed light on the molecular mechanisms underlying the longevity-promoting benefits of bile acids. With the expansion of DA probes to other aging-regulating metabolites, this high-throughput approach will facilitate systematic discovery of aging-regulating mechanisms and pathways.

In parallel, recent advances in limited proteolysis combined with MS[88] (LiP–MS; Fig. 5b) and thermal proteome profiling[89] (TPP; Fig. 5c) have enabled profiling of the metabolite-interacting proteome without probes. By detecting alterations in protein stability following treatment with proteases or heat, these two techniques recognize protein conformational changes occurring upon ligand binding and have been successfully applied to map the metabolite-interacting proteome in *E. coli*[88] and human cells[89]. Currently, interpreting hundreds of enriched protein targets in chemical proteomic data and identifying legitimate targets and regulatory pathways remains challenging. Although methods such as stable isotope labeling with amino acids in cell culture (SILAC) can effectively reduce the false-positive rate in proteomic profiling[87,90], the possibility of DA probe degradation and pulldown of proteins binding the degradation product remains, which can introduce false positives in the identified proteome. For LiP–MS and TPP, factors such as the influence of endogenous metabolites, variation in proteome stability and the precise control of proteases, temperature and treatment time pose additional challenges to obtaining quality data. Thus, validation and functional characterization of chemical proteomics data are necessary for discovery of mechanisms in aging research.

In vivo imaging of aging-regulating metabolites. The longevity-regulating effects of metabolites are dependent on spatiotemporal dynamics, as the tissue/subcellular specificity or availability of metabolites at a given time during development or aging can be determinative for signaling function. For example, the pro-longevity effects of OEA are dependent on nuclear transportation of OEA by the fatty acid-binding protein LBP-8, while regulation of longevity by BCAAs is related to the neuronal effects of these molecules in *C. elegans*[29] and to their availability at different life stages in mice[33–35].

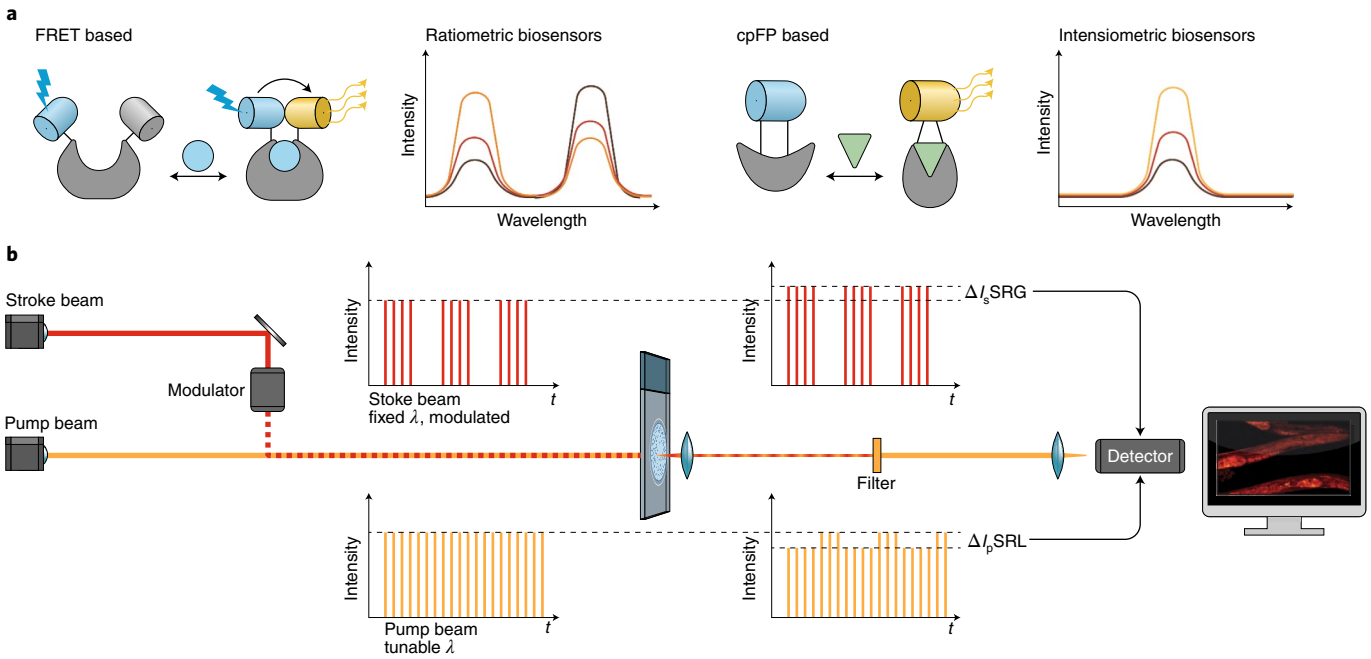

Fig. 6 | Chemical imaging methods for aging-regulating metabolites. a, FRET- or cpFP-based biosensors undergo structural changes following binding of a designated metabolite, which elicits a measurable fluorescence signal corresponding to the concentration of the detected metabolites. **b**, SRS microscopy uses two tunable laser beams (one pump beam and one modulated stroke beam) to detect metabolites with certain chemical bonds in vivo. When the frequency difference between the two laser beams matches the vibrational frequency of a certain chemical bond in the molecule of interest, a stimulated Raman gain or loss (ΔI_sSRG or ΔI_pSRL, respectively) occurs and is captured to detect the molecule at subcellular resolution. With the frequency tuning of the two laser beams, different molecules with distinctive chemical bonds can be visualized in live cells and organisms without labeling.

Thus, the ability to monitor metabolites in vivo is critical for deciphering their signaling mechanisms.

Biosensors are chemical reporters engineered from biological materials, such as RNA, DNA or protein. Because of this, biosensors can be expressed in subcellular compartments or specific tissues, recognizing specific metabolites in live cells and organisms. Periplasmic binding proteins and GPCRs are two protein superfamilies that bind to a variety of metabolites and are often used as recognition elements in biosensors. Following a binding event with a specific target ligand, the conformational changes in biosensors are converted to a measurable signal, such as fluorescence emission, enzymatic activity or transcriptional induction[91]. Fluorescence resonance energy transfer (FRET) fluorescent protein pairs or circularly permuted fluorescent proteins (cpFPs) are common fluorescent elements in biosensor designs (Fig. 6a). For example, BCAA biosensors were developed to generate fluorescence signal changes in a BCAA-concentration-dependent manner[92]. Their application helped reveal the correlation between cytoplasmic and mitochondrial BCAA pools and the impact of extracellular glutamine on intracellular BCAAs[93]. As mentioned above, the effects of BCAAs on lifespan and healthspan seem contradictory between studies, which may be related to variations in supplementation procedures. It will be interesting to apply BCAA biosensors to monitor whether different supplementation procedures result in differences in BCAA distribution among various tissues and affect intracellular BCAA dynamics differently and whether animals at different ages respond to BCAA supplementation in distinct ways. These studies will not only help optimize supplementation procedures for BCAAs to promote longevity but will also advance current understanding of BCAA-regulatory mechanisms, leading the way toward future applications in improving human health. The rapidly expanding toolkit of biosensors provides opportunities to target increasing numbers of aging-regulating metabolites. Future application of these biosensors in aging research holds great promise.

SRS microscopy offers three-dimensional metabolite imaging in vivo. It has the advantage of being able to detect characteristic chemical bond properties in different metabolites and has a diffraction-limited spatial resolution of around 300 nm (Fig. 6b). Application of SRS microscopy has revealed the heterogeneity of different classes of lipid metabolites in lipid particles, such as in comparisons of cholesteryl ester versus triacylglycerol and saturated versus unsaturated fatty acids[94–96]. In particular, an SRS-based imaging method was developed to directly detect retinoids in vivo without labeling or intervention, which revealed an association between induction of retinoids and an extremely long-lived larval stage of *C. elegans* known as dauer[97]. Retinal supplementation improved fat storage during dauer maintenance and increased the lifespan of adult worms under high-glucose stress[97]. Furthermore, integrating deuterium isotope labeling and biorthogonal labeling allows SRS microscopy to trace different metabolites in vivo[95,98], including the longevity-promoting OA and AA. Using SRS microscopy, it will be interesting to track these labeled fatty acids and their metabolites during the aging process and characterize their tissue and organelle specificity in different longevity animal models. Meanwhile, SRS-based genetic screens[99,100] will allow systematic discovery of related factors that mediate the signaling effects of these metabolites. With improvements in detection sensitivity and chemical specificity, future applications of SRS microscopy in aging research will provide both imaging and discovery tools for signaling metabolites and their regulatory mechanisms.

Perspectives. Longevity is a complex multifactorial event that involves modulation by metabolite signals. Decoding the chemical secrets of aging and longevity is intriguing, and the examples we have discussed are just the tip of the iceberg. Many questions

remain, such as how to expand the pool of pro-longevity signaling metabolites and decipher their regulatory mechanisms and how to apply these products of natural chemistry to benefit human health, which are challenging and exciting questions for the field. We look forward to the discovery of more aging- and longevity-regulatory metabolites through metabolomics with advances in AI-aided chemical annotation. Model organisms will continue to be at the forefront in validating the pro-longevity effects of these metabolites and elucidating their underlying molecular mechanisms. Innovative approaches to chemical proteomics and chemical imaging will be integrated with functional genetics and genomics studies and provide powerful platforms for mechanistic characterization. Future studies in this fast-growing area will be instrumental in developing next-generation medications to combat age-related diseases, as well as nutraceutical strategies to improve healthy aging.

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Competing interests

The authors declare no competing interests.

Additional information

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