

# The Gut Microbiota and Healthy Aging: A Mini-Review

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## Abstract

The gut microbiota shows a wide inter-individual variation, but its within-individual variation is relatively stable over time. A functional core microbiome, provided by abundant bacterial taxa, seems to be common to various human hosts regardless of their gender, geographic location, and age. With advancing chronological age, the gut microbiota becomes more diverse and variable. However, when measures of biological age are used with adjustment for chronological age, overall richness decreases, while a certain group of bacteria associated with frailty increases. This highlights the importance of considering biological or functional measures of aging. Studies using model organisms indicate that age-related gut dysbiosis may contribute to unhealthy aging and reduced longevity. The gut microbiome depends on the host nutrient signaling pathways for its beneficial effects on host health and lifespan, and gut dysbiosis disrupting the interdependence may diminish the beneficial effects or even have reverse effects. Gut dysbiosis can trigger the innate im-

mune response and chronic low-grade inflammation, leading to many age-related degenerative pathologies and unhealthy aging. The gut microbiota communicates with the host through various biomolecules, nutrient signaling-independent pathways, and epigenetic mechanisms. Disturbance of these communications by age-related gut dysbiosis can affect the host health and lifespan. This may explain the impact of the gut microbiome on health and aging.

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## The Human Gut Microbiota

The human digestive tract is inhabited by numerous microorganisms. The total estimated number of gut microorganisms is somewhere between  $10^{13}$  and  $10^{14}$ , hovering around the total estimated number of human body cells ( $3\text{--}4 \times 10^{13}$ ) [1]. Bacteria outnumber all other members of the gut microbial community, and the total number of bacterial species found in the gut is estimated to be about 500–1,000 [2]. The most populous bacterial phyla, constituting more than 90% of the gut microbiota are Bacteroidetes and Firmicutes [3]. The remainder consists of many species in other phyla in lower abundance, some

of which may provide important metabolites and functions for healthy aging.

According to the 16S ribosomal DNA sequencing data of fecal samples, individual gut microbiotas show distinct profiles, and this inter-individual variation is greater in older adults [4]. Longitudinally, however, gut microbiotas of healthy adults are relatively stable even for decades [5]. These phylogenetic data are supported by metagenomic analysis of whole shotgun sequencing data: SNP variation patterns show stability over time [6]. Thus, once established early in life (even within 3 years after birth) [7], the gut microbiota seems to be rather stably maintained. Nevertheless, it is responsive to the host's dietary and health conditions [8], much as the host's epigenome is to various environmental cues. In fact, the gut microbiota interfaces the gut environment with the epigenome, but its communication with the host systems involves various signaling networks and their mediators. For instance, the "gut-brain axis" connects the gut microbiome with the central nervous system via neurons, hormones or cytokines [9].

Despite the marked inter-individual variation in the gut microbiota profile, an array of bacterial genes exists that individual hosts share, as shown by functional metagenomics [10]. This "functional core microbiome" (in this review, microbiome denotes the combined genomes of the constituent microbes in the microbiota) is collectively provided by different microbial taxa, indicating that different microbial species can functionally replace one another. The presence of such a core microbiome makes sense if the core functions concern house-keeping or other important biochemical or physiological pathways [11, 12]. In this regard, the core microbiome is likely to consist of omnipresent taxa. Fecal samples collected from different countries over several continents contain 3 distinct microbial metagenomic clusters, designated as enterotypes [13]. These enterotypes are characterized by the most abundant genera in Bacteroidaceae, Prevotellaceae, and Ruminococcaceae families. The first 2 belong to the Bacteroidetes phylum and the last to the Firmicutes. The enterotypes are not correlated with host features such as body mass index, gender, and even age, and this implies the universality of the enterotypes. A separate phylogenetic study found a core microbiota common to 4 different age groups: young (22–48), elderly (65–75), centenarian (99–104), and semi-supercentenarian (105–109) [12]. This core microbiota includes Bacteroidaceae, Ruminococcaceae, and Lachnospiraceae families, the last 2 of which belong to the Firmicutes. Thus, most adult age groups, from young to extremely old, seem

to possess a common core function in their microbiomes that is provided by members of abundant taxa. If so, what is important in the gut microbiota for healthy aging could be a compositional change in the functional core microbiome or an enrichment of non-core functions with advancing age.

### **Changes in the Gut Microbiota Associated with Longevity or Healthy Aging**

One potentially productive approach to the roles of the gut microbiota in human aging is to compile age-related changes in the gut microbiota and examine whether these changes have any biological relevance. Cross-sectional studies of fecal samples from individuals in different age groups suggest age-related changes in the gut microbiota composition and diversity, which concurs with longitudinal study results [4]. In general, the gut microbiota of the elderly becomes more diverse and variable with advancing age [4]. For instance, the 3 bacterial families in the core microbiota, mentioned in the previous section, become less abundant in older age groups, while certain health-associated species become more abundant in older age groups including centenarians and semi-supercentenarians (aged 105–109) [12]. These changes in composition and diversity are also reflected in age-dependent reshaping of co-abundance networks.

Certain changes in composition and diversity are associated with biological or functional age, independent of chronological age. Various measures of frailty have been used as indicators of biological age [14], and gut microbiota composition is associated with biological age [8, 15–17]. Also, gut microbial diversity inversely correlates with biological age but not with chronological age [16, 17]. Furthermore, a co-abundance module consisting of *Ruminococcus*, *Coprobacillus*, and *Eggerthella* genera becomes abundant with an increase in biological age, independent of chronological age (Maffei et al. [17]). The first 2 genera of this module belong to the Firmicutes phylum, and the last one to the Actinobacteria. An interpretation of these results is that as biological age increases, overall gut microbiota richness decreases, while some microbial taxa associated with unhealthy aging emerge. Thus, what happens in the gut microbiota with advancing biological age can be very different from what happens with chronological age, which illustrates the importance of using a biological or functional measure in aging studies.

Even with an age-related change that seems biologically meaningful in hand, it is difficult to determine any

causal roles of the human microbiota in aging. However, the lack of such information has been partially circumvented by data generated from studies using tractable model organisms.

## Animal Models of Gut Microbiota in Longevity and Healthy Aging

### Nutrient Signaling Pathways

Nutrition is the major factor that shapes the host's gut microbiota. It also affects the host's epigenome; for example, folate and choline, as dietary methyl-donors, can affect DNA methylation [18]. Furthermore, nutrition is a key environmental factor that interacts with the host genes, especially those in the nutrient-signaling pathways. Thus, nutrition is a common factor that can link the gut microbiome with the host genome.

Dietary or caloric restriction (CR), a moderate reduction in food intake, extends both health and lifespan [19]. It is a widely conserved intervention that engages biological pathways that are evolutionarily conserved from yeast to primates. However, much remains to be learned. Nutrient availability may not be the only input that can affect the pathways that actuate the CR response because modifications of gustatory or olfactory neurons or even treatment of animals with diet-derived odors can modulate lifespan in *Caenorhabditis elegans* and *Drosophila melanogaster* [20, 21].

The nutrient signaling pathways include the insulin/insulin-like growth factor-1 signaling (IIS) pathway [19]. The activation of AKT (protein kinase B) by nutrient abundance leads to the inactivation of FOXO (a Forkhead box O transcription factor). FOXO is a central factor to longevity, as it induces expression of many proteins involved in cell metabolism, autophagy, and stress-response [22]. Thus, reduced nutrient availability or mutations that weaken the AKT activity result in enhanced FOXO activity. FOXO is abundantly expressed in the *C. elegans* intestine [23], and intestinal FOXO expression alone fully restores the lifespan of FOXO-deficient mutants [24].

Besides FOXO, FKH, a homolog of FOXA (Forkhead box A), is required for the IIS. Gut barrier and nutrient transport functions decline with aging, and dysbiosis weakens the intestinal barrier function, resulting in higher mortality of aged fruit flies [25]. Gut-specific FKH up-regulation improves gut barrier function and increases expression of nutrient transporters in aged flies, resulting in lifespan extension. FKH is also required for the mTOR pathway (see below), thus connecting both pathways [26].

Another important nutrient signaling pathway involves mTOR (mechanistic or mammalian target of rapamycin), which responds not only to nutrients but also to other signals, such as growth hormones and mitogens. As the name indicates, rapamycin targets mTOR to block its signaling pathway for cell growth and proliferation. A serine/threonine kinase, mTOR exists in 2 protein complexes, mTORC1 and mTORC2, as a catalytic subunit in each complex. Both complexes regulate many cellular processes important for cell growth, proliferation, and survival, but some differences exist between them [27]. For example, the effects of mTORC1 seem to be more extensive than those of mTORC2, and the inhibition of mTOR by rapamycin in mTORC1 is more immediate than that in mTORC2. Thus, the anti-aging effects of rapamycin are viewed to be mainly through its effect on mTORC1 [27]. Significantly, the health and lifespan extension by dietary intervention or inhibition of TOR activity by rapamycin involves altered gut microbiota in mice [28, 29].

As mentioned above, the gut microbiome and the host nutrient signaling pathways are interconnected in part because nutrition is the major common factor. A series of experiments using *C. elegans* shows that the relationship between the gut microbiome and the host IIS and TOR signaling pathways goes deep to the functional level to modulate the host's health and lifespan. These worms are grown on media containing a single bacterial strain as a food source, which greatly facilitates sorting out bacterial genes of interest. Han et al. [30] used a collection of *Escherichia coli*-viable deletion mutants as a food source and found 29 bacterial mutants robustly extending the host lifespan. Some of them also lowered the mortality of worms carrying germline tumors or human amyloid- $\beta$  (A $\beta$ ). Interestingly, the lifespan extending effects of many of these bacterial mutants disappeared in the host worms containing mutations that disable the IIS/TOR signaling pathways. This suggests that these bacterial mutants in the gut depend on the host IIS/TOR pathways for their lifespan extending effects. Disturbance of the dependence may well diminish or even reverse the beneficial effects.

### Gut Dysbiosis and Innate Immunity

Changes in microbial composition or outgrowths of certain taxa lead to gut dysbiosis, which refers to the disruption of the commensal homeostasis between the host and the gut microbiota. Gut dysbiosis is associated with many pathological conditions, including inflammatory bowel disease, obesity, diabetes, cardiovascular diseases, and neurodegenerative diseases [9, 31]. Furthermore, a

study using African turquoise killifish indicates that prevention of age-related gut dysbiosis can be conducive to longevity. Smith et al. [32] found that in the killifish, loss of the gut microbial richness occurs during aging, and middle-aged killifish that were treated with antibiotics and then with intestinal contents from young fish showed significant lifespan extension [32]. The long-lived recipient fish maintained higher mobility and microbial diversity compared with controls.

In *Drosophila* intestinal epithelium, deregulated proliferation of intestinal stem cells (ISCs) increases in aging flies, leading to polyploid and poorly differentiated cells [33]. This dysplasia accompanies epithelial malfunctions, such as loss of the intestinal barrier for selective permeability, leading to increased infection and mortality. Curbing the ISC proliferation in old flies extends lifespan [34]. The number of gut microbes increases in older flies [35], and old flies reared axenically (e.g., with antibiotics) show delayed dysplasia [36]. These results suggest that gut dysbiosis most likely underlies the intestine epithelial dysplasia in aging flies.

In *Drosophila*, which lacks adaptive immune function, the primary immune defense is provided by the innate immune response. Two main innate immune pathways are at work in ISCs. One is through activation of dual oxidase that generates reactive oxygen species (ROS) in response to bacterial uracil [37, 38]. The other pathway is through the activation of the immune deficiency (IMD/Relish) pathway, in response to bacteria-derived peptidoglycan, resulting in increased expression of antimicrobial peptides (AMP). The ROS or AMP initiates an immediate immune response against invading pathogens or gut dysbiosis [37, 38].

The gut microbiota of old animals is so dysbiotic that it can initiate an innate immune response. The number of gut microbes increases with age [35], and AMP expression significantly increases in aging flies, likely in response to increasing inflow of microbes. Flies with chronically activated IMD/Relish (nuclear factor- $\kappa$ B) signaling are prone to bacterial infection and lifespan reduction. Modulation of dysplasia and lifespan can be achieved by regulated expression of peptidoglycan recognition proteins in conventionally grown flies but not in antibiotics-treated flies [21, 36]. Thus, the innate immune response, triggered by gut dysbiosis, can affect gut dysplasia and mortality of the host.

The causative role of gut dysbiosis in innate-immunity-induced inflammation becomes convincing in a study using mice. Transfer of the gut microbiota from old mice to young germ-free mice triggers innate immunity and

inflammatory responses mimicking “inflammaging” [39]. These include increased CD4<sup>+</sup> T cell differentiation in spleen, inflammation in the intestine and upregulated expression of inflammatory cytokine genes, such as tumour necrosis factor- $\alpha$ , and increased circulation of inflammatory factors of bacterial origin. Circulation of bacterial compounds in the host is probably due to damage of the intestinal epithelium by inflammation. All these results indicate that the elevated innate immune response caused by dysbiosis in the aging gut can provoke chronic inflammation, leading to gut dysplasia. Gut dysplasia in turn can cause defective epithelial functioning, making the host prone to unhealthy aging, infection, and increased mortality.

#### *The Gut Microbiota in Animal Models of Diseases*

Factors that contribute to gut dysbiosis are linked to the development of pathological conditions. These factors include unbalanced diet, environmental toxins, drugs, ROS, psychological stressors, and other proinflammatory factors. For instance, gut dysbiosis caused by taking antibiotics or high-fat or carbohydrate diet is associated with obesity and metabolic disorders [40, 41]. Causal roles of such dysbiotic factors in pathogenesis can be seen better in animal studies.

Ridaura et al. [42] transplanted fecal matter from twins discordant for obesity into germ-free mice and found that the mice’s body composition measurements vary according to the human donor’s body composition features. The differences in body composition between mice with the obese twin’s microbiota and mice with the lean twin’s microbiota are associated with the differences in meta-transcriptome profiles. These results show a causal role of the gut microbiota in obesity as well as the potential use of the gut microbiota as a therapeutic target.

Another approach to causal relationships between microbes and phenotypes was made using gnotobiotic mice carrying or missing distinct microbial groups [43]. These investigators generated groups of gnotobiotic mice that carry defined microbiotas and exhibit differential survival rates for colitis. By creating hybrid mice with mixed microbiotas that display intermediate susceptibility to colitis, they found the Lachnospiraceae family beneficial. By directly administering suspected members of the family into colitis-prone mice, they found *Clostridium immunis* to be protective against colitis-associated death of the host.

Unlike aged mice fed a low-glycemic diet, those fed a high-glycemic, iso-caloric diet develop many features of age-related macular degeneration (AMD) [44]. Changing

from high- to low-glycemic diet, even late in life, can stop or reverse progression of the AMD features. Low glycemic diets seem to suppress accumulation of several modified molecules, including advanced glycation end products. Furthermore, the severity of retina damage is inversely correlated with the abundance of serotonin, suggesting a protective role of serotonin. In fact, selective serotonin reuptake inhibitors, used as antidepressants, increase serotonin levels in the brain, and people using them are less likely to develop diabetic retinopathy [45]. Most of this mono amine neurotransmitter is synthesized by tryptophan hydroxylase 1 in enterochromaffin cells of the digestive tract. Certain spore-forming gut bacteria, whose identity is yet to be determined, stimulate tryptophan hydroxylase 1 expression and serotonin release from enterochromaffin cells, which seems to be mediated by more than a dozen gut metabolites, including short-chain fatty acids [46]. These results suggest that the effects of glycemic diets on AMD features may involve signaling biomolecules along the gut-brain axis.

The gut microbiota is also causally linked to the development of neurodegenerative disorders [9]. One of the major risk factors for Parkinson's disease is aggregation of  $\alpha$ -synuclein in brain neurons. Mice overexpressing  $\alpha$ -synuclein develop  $\alpha$ -synuclein aggregates and defects in motor function and gut motility [47]. However, germ-free mice overexpressing  $\alpha$ -synuclein show significantly fewer  $\alpha$ -synuclein aggregates, and fecal samples from Parkinson's patients exacerbate motor dysfunction in mice, indicating the causative role of the gut microbiota in  $\alpha$ -synuclein aggregation and the resulting pathology. Furthermore, enteroendocrine cells in the gut epithelium express  $\alpha$ -synuclein, and these enteroendocrine cells are physically close to  $\alpha$ -synuclein-containing enteric neurons, prompting the hypothesis that  $\alpha$ -synuclein originates from the gut and spreads to the central nervous system [48].

Causative roles of the gut microbiota in Alzheimer's disease were studied similarly. Cerebral deposition of A $\beta$  plaques is a critical risk factor for various types of dementia, including Alzheimer's disease. Transgenic mice expressing A $\beta$  precursor protein start to accumulate cerebral A $\beta$  early on, and their gut microbiota composition differs greatly compared with that of the non-transgenic littermates [49]. Transgenic mice rendered germ free show lower A $\beta$  levels and significantly reduced cerebral A $\beta$  deposition compared with conventionally reared transgenic mice. Furthermore, transfer of the gut microbiota from conventionally reared transgenic mice to germ-free transgenic mice was more effective than trans-

fer from wild-type mice in the induction of A $\beta$  pathology. All these observations highlight the importance of the gut brain axis in development of neurodegenerative disorders.

#### *Biomolecules in the Gut Microbiota That Influence Healthy Aging*

Many compounds, either endogenous or exogenous, are known to modulate health and lifespan. One such biomolecule of exogenous origin is rapamycin, which is a natural metabolite produced by soil bacteria. Clinically used as an immunosuppressant, rapamycin binds to its immunophilin, an FK binding protein, and the complex targets mTOR to inhibit its function. In mice, rapamycin alters not only the host gene expression profiles but also the gut metagenomes [29, 50]. Another example of compound of exogenous origin is the diabetes drug metformin. In *C. elegans*, excessive folate limits lifespan [51], and metformin extends lifespan through inhibition of bacterial folate and methionine metabolism [52, 53]. Interestingly, however, altering folate levels of the host has no effect on its longevity [53]. It could be a secondary metabolite generated from bacterial folate metabolism that is responsible for the altered lifespan. Nitric oxide (NO) is a simple yet versatile signaling molecule involved in many physiological functions [54]. It can be produced by the human gut microbiota, but the contribution of the NO generated by gut flora was unknown [55]. A clue was found in a study using *C. elegans*, which cannot produce NO due to lack of the NO synthase. NO provided either by gut bacteria or by exogenous supplementation increases lifespan and stress resistance, demonstrating a profound biological function of NO and the gut microbiota [56].

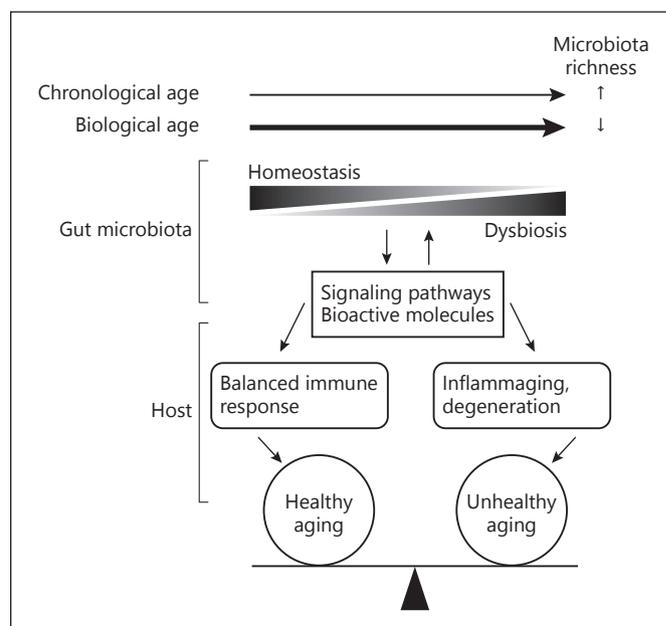
Many biomolecules are produced endogenously by commensal microbes in the digestive tract. These biomolecules include various vitamins, fermentation products, and gut-derived hormones and compounds that are important in neurological health [9]. Colanic acid, either bacteria-derived or exogenously added, extends lifespan in *C. elegans* and *D. melanogaster* [30]. Colanic acid is an exopolysaccharide composed of a repeating unit of sugar monomers that is extracellularly secreted by bacteria. The beneficial effects of colanic acid are independent of the CR signaling pathways. Colanic acid promotes mitochondrial fission and enhances the mitochondrial unfolded protein response under stress conditions.

Of the fermentation products, short-chain fatty acids have profound effects on the host [9]. Short-chain fatty acids are products of the breakdown of dietary fibers by

the anaerobic gut microbiota. They can easily enter the circulation from the gut and have beneficial roles in energy metabolism. Acetate can reduce serum cholesterol and triglyceride levels, propionate can lower glucose levels, and butyrate can increase insulin sensitivity in mice. On the other hand, short-chain fatty acids can have harmful effects. In the mouse model of Parkinson's disease mentioned above [47],  $\alpha$ -synuclein aggregates activate immune cells, including phagocytic microglial cells in the CNS, in a gut-microbiota dependent manner. Treatment of germ-free mice overexpressing  $\alpha$ -synuclein with a mixture of acetate, propionate and butyrate leads to neuroinflammation and motor deficits. These results indicate that short-chain fatty acids constitute the main causal factor for the  $\alpha$ -synuclein-related pathology caused by the gut microbiota. Age-related changes in the metagenomics of short-chain fatty acid production have been observed. For instance, frequencies of genes encoding short-chain fatty acid production and those involved in carbohydrate breakdown decrease, while those of genes involved in protein breakdown increase [57]. The reduced frequency of genes for short-chain fatty acid production is also associated with frailty [8]. Thus, the short-chain fatty acids have the potential to modulate healthy aging.

Short-chain fatty acids seem to have both negative and positive effects on health, and to understand this complexity, at least 2 things need to be considered. Physiological responses to input doses are often nonlinear (e.g., U-shaped or J-shaped). To take extreme examples, inactivation of FOXO shortens lifespan, so does its chronic activation [36]. Similarly, chronic activation of IMD/Relish or inactivation of dual oxidase results in shortened lifespan in flies [36, 37]. This indicates that given a dose-response relationship, there is an optimal dose range for the best desired response. The other thing to consider is that the overall effect of a global factor is likely to be the net effect (balance) of all the positive and negative effects combined. Thus, FOXO, a transcription factor regulating expression of many proteins, is an important factor for healthy aging and lifespan of model organisms; given muscle metabolism, however, the activation of FOXO by HDACs can cause skeletal muscle atrophy in mice, probably via autophagy [58], and inactivation of HDAC by butyrate can reverse the atrophy (see below) [59].

Of the primary short-chain fatty acids, butyrate, like trichostatin A, inhibits histone deacetylases (HDACs) and thus has a profound effect on the host epigenome [60]. HDACs catalyze removal of the acetyl group from lysine residues in histones, leading to heterochromatization and transcription repression. Thus, inhibition of



**Fig. 1.** Biological age-dependent gut dysbiosis and unhealthy aging. Biology of aging is better approached using a functional measure of age. An increase in chronological age (in the direction of the arrow) is associated with an increase in phylogenetic richness of the gut microbiota, but an increase in biological age shows an inverse association. As biological age increases, homeostasis of the gut microbiota with the host decreases, while dysbiosis increases. The dysbiotic changes are communicated to the host through various signaling pathways and bioactive molecules, which either delays or promotes proinflammatory immune responses and age-related degenerative pathologies.

HDACs by butyrate promotes histone lysine acetylation, leading to an open chromatin state and transcription activation. Butyrate increases lifespan in *Drosophila* [61]. In aging mice, butyrate counters muscle atrophy [59], as does activation of FOXO by HDAC1 [58]. Butyrate also enhances memory functioning of aged mice [62]. These results demonstrate that relatively simple organic compounds, such as butyrate, can have far-reaching systemic effects on healthy aging.

## Summary

With advancing chronological age, the gut microbiota becomes more diverse, increasing in phylogenetic richness (Fig. 1). However, when biological age is considered with adjustment for chronological age, overall richness decreases, while certain bacterial taxa associated with unhealthy aging thrive. Thus, as biological age increases, the

homeostatic relationship between the gut microbiota and the host deteriorates, while gut dysbiosis increases. These dysbiotic changes in the aging gut can negate the beneficial effects of the gut microbiome on the nutrient signaling pathways, and provoke proinflammatory innate immunity and other pathological conditions. Gut dysbiosis can also disturb the communication between the gut microbiota and the host through various biomolecules, CR-independent signaling pathways, and epigenetic mechanisms, affecting host health and longevity.

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## Disclosure Statement

The authors have no conflicts of interest to declare.

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