C. elegans - A biosensor for host-microbe interactions

Revealing complexity in uncharted functional landscapes

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Abstract

Microbes are an integral part of life on this planet. Microbes and their hosts influence each other in an endless dance dictating how the meta-organism interacts with its environment. While great advances have been made in microbiome research over the past 20 years, the mechanisms by which both host and their microbes interact with each other and the environment are still not well understood. The nematode *Caenorhabditis elegans* has been widely used as a model organism to study a remarkable number of human-like processes. Recent evidence shows that the worm is a powerful tool to investigate in fine detail the complexity that exists in microbe-host interactions. By combining the large array of genetic tools available for both organisms together with deep phenotyping approaches, it has been possible to uncover key effectors in the complex relationship between both parts. In this review, we survey the literature for insightful discoveries in the microbiome field using the worm as a model. We discuss the latest conceptual and technological advances in the field, and highlight the strengths that make *C. elegans* a valuable biosensor tool for the study of microbe-host interactions.

Introduction

Microbes are a major force in nature that have shaped this planet since the origin of life[1]. Animals and plants have been in close contact with microorganisms for billions of years, affecting each other's biological functions that ultimately carved their evolutionary trajectories[2–4]. This shared history led to the acquisition of dependence between the two biological systems, microbes and hosts, that range across several taxonomic levels[2,4]. The extensive variety of interactions between the host and its microbes raises challenges to our understanding of what an organism or a biological function is in the context of the microbiome[5–7], and defies the very same notion that all animals need a microbiome[8]. Nevertheless, microbial communities are key drivers regulating a wide range of biological functions, affecting nutrition, immunity, and many other processes[9]. Yet we are still far from achieving a complete understanding of the intricate relationship between the host and its microbiota. The inherent complexity of this problem can be tackled from different angles using a wide array of tools including theoretical approaches[10–12], classical *in vitro* studies[13,14], and the use of animal models[15,16]. The latter offers the possibility to establish causality links, granting robust interpretations of the real influence of one system upon the other. An increasing number of animal models are currently being used to tackle this problem. In particular, the use of *Caenorhabditis elegans,* a bacterivore nematode, has emerged as a very suitable model that offers strong benefits over its limitations[17–19].

In this perspective, we highlight the reasons that make *C. elegans* a supreme model for the study of microbe-host interactions by focusing on the methodologies available and the insightful discoveries made over recent years using the worm as a biosensor of microbial activity.

The worm and its microbiota – Parallels to humans

From an evolutionary point of view, *C. elegans* is a suitable model for the identification of the molecular processes involved in pathogenic and commensal interactions, as the mechanisms involved are often conserved in other organisms of interest, including humans[20–22]. At the level of the gut, basic morphological similarities exist between the intestinal cellular structure in humans and worms. Added to the functional similarities in the extraction and absorption of nutrients and the ability to host live microbes, the gut of the worm is a perfectly adequate small but physiologically relevant organ for the analysis of host-microbe interactions. In the wild, *C. elegans* harbours a rich and diverse microbiome that is relatively stable across geography. Like mammals and other animals, the microbiota of *C. elegans* is composed of the major phyla such as Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria[23–25], the latter being often the most dominant, mostly from the *Enterobacteriaceae* genus. Likewise, studies have shown great intra-species variations of the intestinal microbiota which varies according to the environment as well as the host's genetics[19,25,26]. For example,

Gammaproteobacteria such as the human pathogen *P. aeruginosa* exploit monosaccharides from the intestinal mucin layer to successfully colonise *C. elegans*[27]. Whether other commensal, pathobionts and/or pathogenic microbes explore this mechanism for worm colonisation remains to be studied. Historically, the most commonly used bacteria in the context of *C. elegans* work in the laboratory setting has been mono-colonisation with the enterobacteria *E. coli*[28,29], an important representative of a human gut commensal. Like mammals, *C. elegans* acquires an active gut microbiota through the oral route where bacteria serve many roles beyond providing nutritional sustenance[24]. The view that bacteria serve as nutrients only for worms but not for mammals is also misconception. As found in worms, microbes from the environment are often present in the distal gut of mammals[30]. Similarly to worms, bacterial contents are constantly released into the mammalian gut as one third of the bacterial cells are damaged with impaired membrane polarity and approximately one quarter with severely compromised membrane integrity[31]. Hence, several bacterial products can be found in the host bloodstream such as lipopolysaccharides (LPS)[32], gut-commensal proteins[21] and a wide range of metabolites are either unique to bacteria or co-metabolites of unknown origin. Similar to mammals, a vast literature shows that bacteria supplement the worm host with metabolites that regulate many physiological and metabolic traits or regulate the effects of drugs or nutrients, through mechanisms that are independent of the macronutrient content of bacteria[24,25,29,33–38]. Therefore, the recent focus on commensal interactions joins past findings where *C. elegans* has been an instrumental workhorse to study microbial pathogenesis[39–42]. As new resources become available (e.g. CeMbio) a larger array of bacterial species can now be used to form complex communities covering the major phyla and mimicking the microbial environment in the gut of other organisms[2,10,43]. The utilisation of a phylogenetically and metabolically diverse microbial community makes it a suitable model to study functional aspects that are equally present in the human gut microbiome[9,33,44,45]. Interestingly, work in both humans and other organisms including *C. elegans* suggests that the functional capability of the microbiome along with its phylogenetic composition, are a good proxy to link microbial community composition and its regulatory effects on the host[5,26,46,47]. This advocates for a need to characterise microbiome function with high precision to fully capture its mechanistic links in modulating host molecular and physiological phenotypes.

Insights into host-microbiota interactions using worm phenotypes as readouts of bacterial activity

Several reports using *C. elegans* as a model organism published over the last 10 years have provided remarkable insights into host-microbe interactions (Figure 1). For example, several studies using nematode development as a phenotypic readout have shown that its microbiota is essential for the supply of micro-nutrients such as vitamin B2[48], B6[29], B9[29,49], B12[50,51], iron[21,31,52] and molybdenum[36],

as well as reactive oxygen species[31,53]. The *C. elegans* model has also proven useful in identifying other metabolites at the interface between microbes and host, which regulate adult physiological traits. In particular, *C. elegans* adult survival and longevity phenotypes have identified the production of nitric oxide[22,54,55] by the Firmicutes *B. subtilis*, and colanic acid[30,56,57], methylglyoxal[58], folate[49,59] and agmatine[28] from the Proteobacteria *E. coli* as key molecules regulating host ageing. Moreover, perturbation of bacterial respiration through coenzyme Q biosynthesis impairment leads to modulation of *C. elegans* lifespan[60,61]. Using host lipid metabolism as a phenotypic readout, *C. elegans* has also allowed the identification of microbial metabolites that regulate host lipid metabolism through NR5A-Hedgehog signalling[62]. In addition, *C. elegans* has been used as a biosensor to conduct studies on probiotics[63]. In a recent study, Goya and colleagues found that using a *C. elegans* synucleinopathy model, *B. subtilis* PXN21 inhibits, delays, and reverses α-synuclein aggregation causing Parkinson's disease through alterations in the sphingolipid metabolism pathway of the host[64]. Additionally, Urrutia and colleagues evaluated the effect of various bacteria on another *C. elegans* model of neurodegeneration and found that bacterial production of GABA and lactate conferred 40% of neuroprotection[65]. These studies provide links between bacteria and brain pathologies, offering further mechanistic insights into how the gut microbiota regulates motor deficits and neuroinflammation that were observed in a mouse model of Parkinson's disease[66]. However, the mechanistic basis of this microbiota–brain signalling and its physiological relevance remain largely unknown. Using *C. elegans* olfactory responses (e.g. attractions to odours) as a phenotypic readout, it has been shown that commensal gut bacteria alter olfactory behaviour through the production of the neuromodulator tyramine. In particular, production of this metabolite by commensal *Providencia* bacteria bypasses the requirement for host tyramine biosynthesis and manipulates a host sensory decision promoting fitness of both the host and the microorganism[67]. Likewise, the ability of *C. elegans* to "read" and recognise bacterial small RNAs induces a transgenerational response leading to appropriate behavioural changes in their progeny for pathogen avoidance[41]. Further, the use of *C. elegans* as a biosensor has highlighted that the microbiota has a fundamental role in defense of the host against pathogens[68–71] – a mechanism known as colonisation resistance[2]. In particular, using worm survival as a physiological readout, *C. elegans* allowed the identification of cyclic lipopeptides produced by the microbiota that confer resistance against intestinal colonisation by the human pathogen *P. aeruginosa* and by natural pathogens of the worm such as *B. thuringiensis*[59].

In addition to identifying key mechanisms and metabolites involved in microbe-host interactions, the worm has also been a great model for revealing novel host mechanisms. For example, it reveals the molecular processes on the host that interprets signals from bacteria which play an essential role in mediating various physiological functions. A groundbreaking study by Liu and colleagues identified the

organismal pathways that survey and defend mitochondria against toxic by-products of several members of the microbiota[72]. Another example which illustrates this connection is the production of biofilm by *B. subtilis* leading to increased life expectancy of the worms. This is the result of the production of communication molecules involved in bacterial quorum sensing and nitric oxide (NO). These molecules then trigger a dietary restriction-like response which involves the DAF-2, DAF-16 and HSF-1 signalling pathways that regulate lifespan[54,55]. Additionally, several bacterial metabolites have been shown to impact host lifespan and healthspan by acting on mitochondrial function, activating the unfolded protein response, remodeling the host lipid response, and interfering with insulin-like and dietary restriction-related pathways[73–75].

Over the years, the worm has provided unique insights into health and disease phenotypes by elucidating key molecular mechanisms occurring in both entities of this complex meta-organism.

An experimental pipeline to explore mechanisms of host-microbe interactions

Interactions in the host-microbe system are a two-way road, where each part is sensing and reacting to signals from its counterpart in a continuous loop. Interventions in the host and/or its microbes transforms how the entire system reacts to stimuli coming from a set of environmental conditions. Being able to characterise how both microbes and host perform these tasks is therefore essential to capture the mechanisms underlying their interaction. Molecular and synthetic biology tools to modify and study with great precision most layers of biological information exist for the worm host, the microbial community, and its environment (Figure 2). Thus, the worm and its microbiota provide an excellent system where each variable can be modified while concomitantly allowing for deep phenotyping[76].

1) The *C. elegans* host

This semi-transparent nematode has a short life cycle (approximately 3 days) and lifespan (mean of approximately 18 days), that together with its simple and cost-effective handling allows the study of a wide range of evolutionarily conserved processes and diseases in humans[77]. The superpower of the worm model lies in its simple genetics and amenability for high-throughput screening. As fully reviewed in [78], "The power of any genetic model organism is derived, in part, from the ease with which gene expression can be manipulated". Therefore, *C. elegans* is a great genetic model organism due to the wide range of molecular tools available to modify its genome, the low cost and simplicity associated with generating mutants and a fully supportive community of researchers that widely distribute their reagents. For example, thousands of genetically modified strains are

readily available from the Caenorhabditis Genetic Center and the National BioResource Project Lab. The creation of mutant strains is also very well documented, both for the knockout and knockdown of genes and the generation of transgenic lines[78–81]. Precisely regulating the expression of host genes is essential for causally linking the influence of an environmental cue with a phenotype. In particular, unbiased forward (e.g. random mutagenesis[82–85]) and reverse genetic screens[79,86] have been powerful tools in revealing novel genes linking genotype to phenotype. For example, in the context of host-microbe interactions, an RNAi-based approach has provided remarkable insights into the host mechanisms regulating microbial aversion[87]. The transparent nature of worms allied to the simple generation of transgenic fluorescent reporter lines permits the study of how microbes regulate gene expression profiles in a spatial (tissue-specific), temporal and quantitative manner[88]. The combination of these unique features and molecular techniques has been employed to identify microbial signalling and metabolic pathways, including the respective metabolites, regulating host lipid metabolism[28]. Furthermore, the combination of these approaches with forward and reverse genetic methods has been instrumental in identifying the host genetic networks underlying lipid metabolism regulation mediated by bacterial vitamin B12[51]. Overall, these tools allow researchers to modify and study with precision the role that host genetics plays in determining a molecular or physiological phenotype driven by microbes.

C. elegans provides an excellent opportunity to perform microbiota studies at a scale only outcompeted by *in vitro* unicellular high-throughput screening[17]. For example, several worm phenotypes can be monitored in a high-throughput manner. This includes survival readouts when worms are challenged with pathogens or xenobiotic compounds[20,39,40,68,89–93], measurements of adult lifespan[30,32,54,55,57,64,70], arrested development[21,22,32,36], impaired fertility[54,55,69,94,95] resistance to stress[55,70], and altered behaviour[96,97]. The measurement of such phenotypes in a high-throughput manner allows the study of individual or communities of microorganisms that colonise its intestine in regulating several traits of host physiology. Recently, the high-throughput capacity of this model has experienced a giant leap in the current state of the art - deep phenotyping[76]. This has been possible through a new set of hardware and software tools that allow the manipulation, recording and imaging of worms grown in hundreds of conditions in parallel. These include the use of flow cytometry-like stations as COPAS[98] to measure worm physical variables such as axial length and optical density; the 'WorMotel' from Fang-Yen[99] and the Lifespan Machine from Fontana[100] that measure lifespan; diverse tracking platforms from Brown[96,101], Nollen[102], Rex[103], Driscoll[104] or Goodman[105] labs that can assess motor-related phenotypical variables including behavior; or fully automated workstations from Pincus[106] or Lu[97] that monitor long-term behavior and healthspan, among many other examples[107–110]. These technologies allow worms to be phenotypically characterised with a high degree of

reproducibility by often affordable imaging systems[111]. Exploring the transparent nature of *C. elegans*, fluorescence imaging can also be performed in a high-throughput manner, further extending the screening capabilities of the worm as a biosensor. Fluorescence information can be obtained at different scales, from studying protein dynamics in whole animals[108,112] to processes in particular specific cell lineages[113]. Hence, by using worm molecular or physiological phenotypes as readouts of bacterial activity, it is possible to capture in fine detail several molecular mechanisms at the host level involved in these complex host-microbe interactions. As a consequence, this leads to the generation of a large amount of complex phenotypic data which requires the use of computational tools to analyse and extract meaningful information. To solve this issue, machine learning and deep learning algorithms are now being used in addition to commonly used statistical techniques (e.g. PCA or correlation[114]) to uncover hidden features and trait prediction[115] from complex datasets.

Thus, *C. elegans* provides unique opportunities as a model organism for studying host-microbe interactions, as several layers of information can be edited and studied at a depth covering a vast and uncharted functional landscape.

2) The microbes

A major problem of current microbiome research is the excess of data available implying causation between microbiota physiology and host function drawn simply from correlation data. One potential solution to this problem is the creation of synthetic communities which may offer a plausible strategy to dissect causality in complex host-microbiota interactions. To increase the likelihood of success one should consider creating a simplified microbial community that represents both phylogenetically and functionally the complex microbiome that it is attempting to represent. It should also be designed taking into consideration the model organism that will host the community. Finally, and most importantly, whether the mock-community and their host are adequate models to address the scientific question at hand. The groups of Félix, Samuel, Schulenburg and Shapira[23–25] have importantly contributed towards this goal. As a result of their in-depth meta-analysis of the natural microbiome of *C. elegans*, they have created CeMbio[43] - a simplified natural worm microbiota mock-community. Some of its key features include a set of strains easily culturable that colonise the worm gut and that distinctly affect *C. elegans* physiological traits and life trajectories. These strains have fully sequenced genomes, diagnostic PCR primers and characterised metabolic network models allowing modelling with ease. Computer modeling of metabolism and experimental characterisation of bacterial physiology allow to study not only the impact of bacterial functional diversity but also the role of the environment on host functions. Zimmermann and colleagues have led this integrative approach, bringing together the use of phenomic microarrays (Biolog) technology[116], to assess

metabolic competences of selected bacteria, metagenomics and computer modeling, to reconstruct metabolic networks at the community level and study ecological interactions between members of the community. Their work has shown that host physiology and fitness is dependent on the nutritional landscape for microbe-microbe interactions[117].

Yet, to functionally characterise the contribution of each microbe in maintaining the homeostasis of the community and their role in regulating host physiology, one needs to go deeper in the functional characterisation of each microbial member. For this purpose, the availability of a wide range of technologies for bacterial modification is necessary, such as the random insertion of transposons in the genome[34,118–120] and the use of bacteriophages[35]. These techniques allow the creation of mutant libraries from several species giving us an opportunity to study and identify new genes regulating bacterial function. For example, such approaches have led to the identification of thousands of bacterial genes belonging to Proteobacteria and Bacteroidetes of previously unknown functions[121]. One of the most notorious examples is the construction of the Keio library, which contains a set of precisely defined monogenic deletions of all the non-essential genes of *E. coli* K-12 (3,985 genes out of a total of 4,288)[122]. These transposon and deletion bacterial libraries are now being used in mono-colonisation experiments to causally link the effects of bacterial genes from a single species with a wide range of phenotypes from diverse hosts[51,123–127]. For *C. elegans*, the Keio library has been screened for *E. coli* genes involved in the regulation of lifespan[30,49], to show the dependence of the host development on its microbiota for micronutrients (e.g. folate, iron and molybdenum[31,32,36]) and to infer the role of the microbiota in drug action[29,128] (e.g. fluoropyrimidines). *B. subtilis,* a human probiotic bacterium, is another commonly used bacterial strain for mono-association studies with *C. elegans*. The Gross lab has constructed two ordered, barcoded, antibiotic-resistance-marked single-gene deletion libraries, comprising 3968 and 3970 genes respectively, allowing the study of gene and pathway function genome-wide of a Gram-positive bacteria on host physiology[129]. To date, this resource has not been used in combination with a *C. elegans* host but may well provide an important tool to expand the possibilities of this microbiome model beyond its current potential. While loss-of-function libraries are more widely used and studied with *C. elegans*, gain-of-function libraries are also available. For example, the ASKA library, made up of single strains containing multicopy vectors overexpressing any gene of *E. coli*[37], was used with *C. elegans* and led to the identification of the bacterial metabolite methylglyoxal in regulating host lifespan[58].

New tools are also being developed or implemented to study the gut environment in *C. elegans.* For example, RNAseq has been used to study the effects of the gut environment and genetics of *C. elegans* in bacterial gene expression and metabolic pathways of *E. coli* inside the gut[130]. The authors found that active metabolism of bioactive lipids in the gut may regulate potential host-

microbial interactions. A similar observation was recently made in a mammalian model, where sphingolipids produced by the microbiota enter and regulate host lipid metabolism[131]. Interestingly, the authors observed an increase in aerotaxis-related genes by bacteria growing in the gut compared to *in vitro* growth, suggesting that the gut of *C. elegans* may in fact be anaerobic. While this is an interesting observation, which could further expand the usefulness of this model, measurements of oxygen tension inside the worm gut are required. New synthetic biology microbial reagents are also being designed to expand this toolset. This includes the development of bacterial biosensors capable of detecting molecules in the gut of worm[132], bioluminescent bacteria to evaluate bacterial survival in the gut[133], and the development of optogenetic tools in bacteria to control bacterial metabolism and indirectly regulate host physiology[57].

3) The environment

Recent research in humans shows that the environment dominates over host genetics in shaping the microbiota[134,135]. All the aforementioned tools provide a robust framework where the role of the microbiota on host physiology can be captured at the mechanistic level. The scalability of the current worm methods allows the set-up of systematic studies where environmental perturbations can be added as important variables of investigation.

The inclusion of drugs as an additional factor produces a complex interaction landscape between microbes, drugs and host. For example, host physiology may be affected as a result of modified drug pharmacokinetics through direct microbial biotransformation [38,136–138], or alternatively by the indirect effects resulting from the action of drugs on microbial community structure and function[13,14,38,139]. Levodopa, a medicine to treat Parkinson's disease, is used as a carbon source by bacterial taxa containing tyrosine decarboxylase activity, thus reducing its efficacy[140]. For the latter, examples in cancer research show that chemotherapy treatments often lead to intestinal disorders following an overall reduction in microbial abundance[141] or through increased drug toxicity after re-activation by microbial enzymes[142,143]. In addition, drugs can limit the biological functions of some taxa, arresting their growth and allowing other disease-associated taxa to out-compete. For example, colonisation by *C. difficile* is prevented by colonisation resistance properties of the faecal microbiota. Thus, weakening microbial colonisation resistance by widespread use of antibiotics in the clinical setting is a major risk factor for *C. difficile* associated morbidity[144]. *C. elegans* offers a reliable platform to investigate these complex relationships between host, microbes and drugs. Using the worm as a biosensor for host-microbe-drug interactions showed that doxorubicin, a commonly used anticancer drug, is metabolised by human gut bacteria such as *K. pneumoniae*, *E. coli* and *R. planticola* among others[137]. Fluoropyrimidines (5-FU) are essential anti-cancer chemotherapy for colorectal cancer with

highly dependent patient efficacy. To investigate the role of microbes in anti-cancer drug toxicity, our group in parallel with the Walhout and O'Rourke labs developed a three-way (microbe-drug-host) high-throughput screening method to explore the role of microbial genetics in the toxicity of fluoropyrimidines on *C. elegans*[29,128,145] development, reproduction and survival. The relative contribution of each *E. coli* gene in mediating drug toxicity on the host was obtained to perform a genome-wide systematic analysis of the pathways and processes involved in the mediation of drug effects. These three independent studies show that microbes can bolster or suppress the effects of fluoropyrimidines through metabolic drug interconversion involving bacterial vitamin B6, B9, and ribonucleotide metabolism, and highlight the value of these approaches to unravel the mechanistic complexity that exists in such interactions.

Nutrition is a key element at the interface between microbes and host dictating the fitness of the entire meta-organism. Given the immense complexity of nutrition[146], mapping the biological response of a host and its associated microbes to the different types of chemical components is a challenging task. Historically, the microbial nutritional landscape has often been studied with the well-established microbial phenotyping technology from Biolog[116]. This technology allows the investigation of hundreds of metabolites covering all major nutrient classes (e.g. sugars, fatty acids, amino acids) on regulating microbial growth phenotypes. Zimmermann and colleagues recently applied this technology to investigate how different microbes from the natural microbiome in *C. elegans* metabolised a diverse range of nutrients[117]. They showed that specific nutritional requirements by members of the worm's microbiota dictate the nature of their interaction (e.g. competitive, commensal) in a complex microbial community and their role in regulating worm physiology. Using the same technology, our lab developed a high-throughput four-way microbe-drug-nutrient-host screening approach to investigate how 337 dietary elements affect the efficacy of metformin on host physiology in a bacterial-dependent manner. Metformin is the most widely used drug for type 2 diabetes and a potential treatment for ageing or age-related disease. Research spanning from worms to humans shows that metformin acts indirectly through the microbiota to regulate distinct host phenotypes and diseases[147–150]. Using a nutrient systems approach, we discovered that *E. coli* integrates signals from both metformin and the diet into a signalling cascade that affects the expression of the master nutrient regulator Crp, which in turn, indirectly regulates host physiology through modified arginine-derived metabolites[28]. Recently, a study by the O'Rourke lab investigated the role of amino acids in microbe-drug-host interactions to discover that dietary serine enhances fluoropyrimidine anticancer chemotherapy without altering pro-drug activation by *E. coli*[145]. Overall, the current use of *C. elegans* as a biosensor of bacterial activity is one of the ultimate state-of-the-art

models to reveal novel mechanisms at the interface between drugs-nutrients-microbes and host physiology.

Future outlook

The vast complexity present in the human microbiome may only be fully understood with careful and systematic navigation of all the potential physical and biological constraints that exist in the gut. For example, this model can be further extended to probe the effect of a wide variety of environmental conditions including new drugs/xenobiotics or pH fluctuations. As scalability of this system continuously grows from additional technological, biological, and computational tools with seamless integration, new layers of complexity will be captured. Yet, despite the important contributions achieved using this model, the microbiome *C. elegans* research field is still in its infancy. To conquer the vast unexplored complexity that exists in host-microbiome interactions, *C. elegans* research boundaries will have to be expanded to include larger microbial communities in complex but defined nutritional environments. The immense value of mimicking specific human microbiome conditions through the addition of further layers of complexity to this highly scalable system will permit the discovery of key principles in host-microbe interactions.

Past research on *C. elegans* as a model for the study of host-microbe interactions gives us hope. It has permitted the discovery of many bacterial effectors on host physiology and the respective host mechanisms. In particular, some of the most exciting discoveries using worms as biosensors of complex phenotypic traits mediated by microbes have been extended to diverse host organisms[28,56], and identified mechanisms that are conserved across taxa. The quote “You have evolved from worm to man, but much within you is still worm” by the German philosopher Friedrich Nietzsche has often been used to capture with simplicity the use of *C. elegans* as a valuable model organism for human disease processes. And once again, now in the study of complex host-microbe interactions, this simple model organism continues to enlighten and surprise us. What the future holds will lead to pioneering discoveries in one of the most extraordinary and complex problems that biology faces today.

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FIGURE LEGENDS

Figure 1. *Timeline of research publications providing remarkable insights into C. elegans-microbe interactions.* Each publication entry has been classed (by position and colour) regarding the main functional landscape explored in the work. Worm as a biosensor reveals links between: (black) host and/or microbial genetics; (red) drugs-microbe and host; (green)nutrition-microbe and host; (blue) microbe-microbe host relationships. Selected publications have been highlighted in bold.

Figure 2: *An experimental pipeline to explore host-microbe interactions.* A modular, scalable, layered and flexible workflow to explore the complex landscape of host-microbe interactions. Green - Multiple experimental options exist for *C. elegans* molecular or phenotypic readouts, including the use of large mutant libraries for host genetics. Blue - High-throughput screening of single or complex microbial communities and metabolic network modeling tools are available for worm-microbiota studies. Red - Drug and dietary interventions can be incorporated to study the influence of environmental cues on host-microbe interactions. Black - Multi-omics and computational analyses can be applied to individual or combined entities of the worm-microbe model for in-depth molecular characterization. Orange - The evolutionary conserved nature of the worm-microbe model permits testing and further validation of findings in more complex models.

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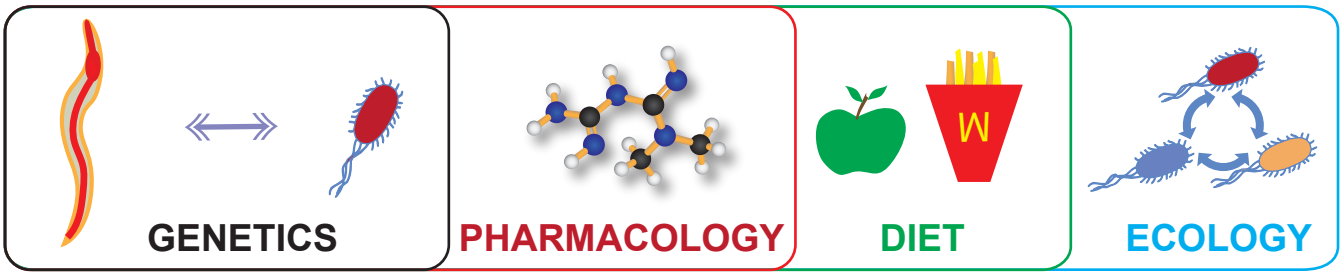
Functional Landscape



Timeline

2002
Bacterial respiration modulates lifespan (Saiki *et al.*[60]; Larsen *et al.*[61])

2008

2012
Bacterial folate depletion and anti-folate drugs extend lifespan (Virk *et al.*[59])

Probiotics protects against pathogens (Montalvo-Katz *et al.*[69]; Kim *et al.*[71])

2013
Metformin extends lifespan through bacterial effects (Cabreiro *et al.*[147])

Bacterial nitric oxide extends lifespan (Gusarov *et al.*[55])

Host genetic network to bacterial aversion (Melo *et al.*[87])

Host genetic metabolic network to bacterial metabolism (Watson *et al.*[74])

Bacteria communicate with the host mitochondria (Liu *et al.*[72])

2014
Interspecies B12 regulates host development (Watson *et al.*[51])

2015
Bacterial ROS metabolism regulates host development (Govindan *et al.*[123])

2016
***E. coli* screen identifies bacterial folate regulating lifespan** (Virk et *al.*[49])

The worm microbiome (Dirksen *et al.*[23]; Samuel *et al.*[24]; Berg *et al.*[25];)

2017
Diet regulates microbial metabolites impacting host lipidome (Lin *et al.*[62])

Bacterial colanic acid extends lifespan (Han *et al.*[30])

Bacterial CSF extends lifespan (Donato *et al.*[54])

First 3-way screens: Bacterial genetics determine cancer drug effects (Scott *et al.*[29]; Garcia-Gonzales *et al.*[128])

Bacterial B2 regulates nutrient absorption (Qi *et al.*[48])

2018
Transformation of the Anticancer Drug Doxorubicin by bacteria (Yan *et al.*[137])

Bacterial siderophores modulate development (Qi *et al.*[21])

2019
Microbiota protects against infection via a cyclic lipopeptide (Kissoyan *et al.*[68])

First 4-way screen: A signalling pathway in bacteria regulates drug effects in lifespan (Pryor *et al.*[28])

Bacterial iron regulates host development (Zhang *et al.*[52])

Regulation of microRNA machinery and development by interspecies S-nitrosylation (Seth *et al.*[22])

Interkingdom transport of molybdenum acts in sulfite detox (Warnhoff *et al.*[36])

Functional repertoire of the worm microbiota (Zimmerman *et al.*[38])

2020
Gut bacteria modulate behaviour (O'Donnell *et al.*[67])

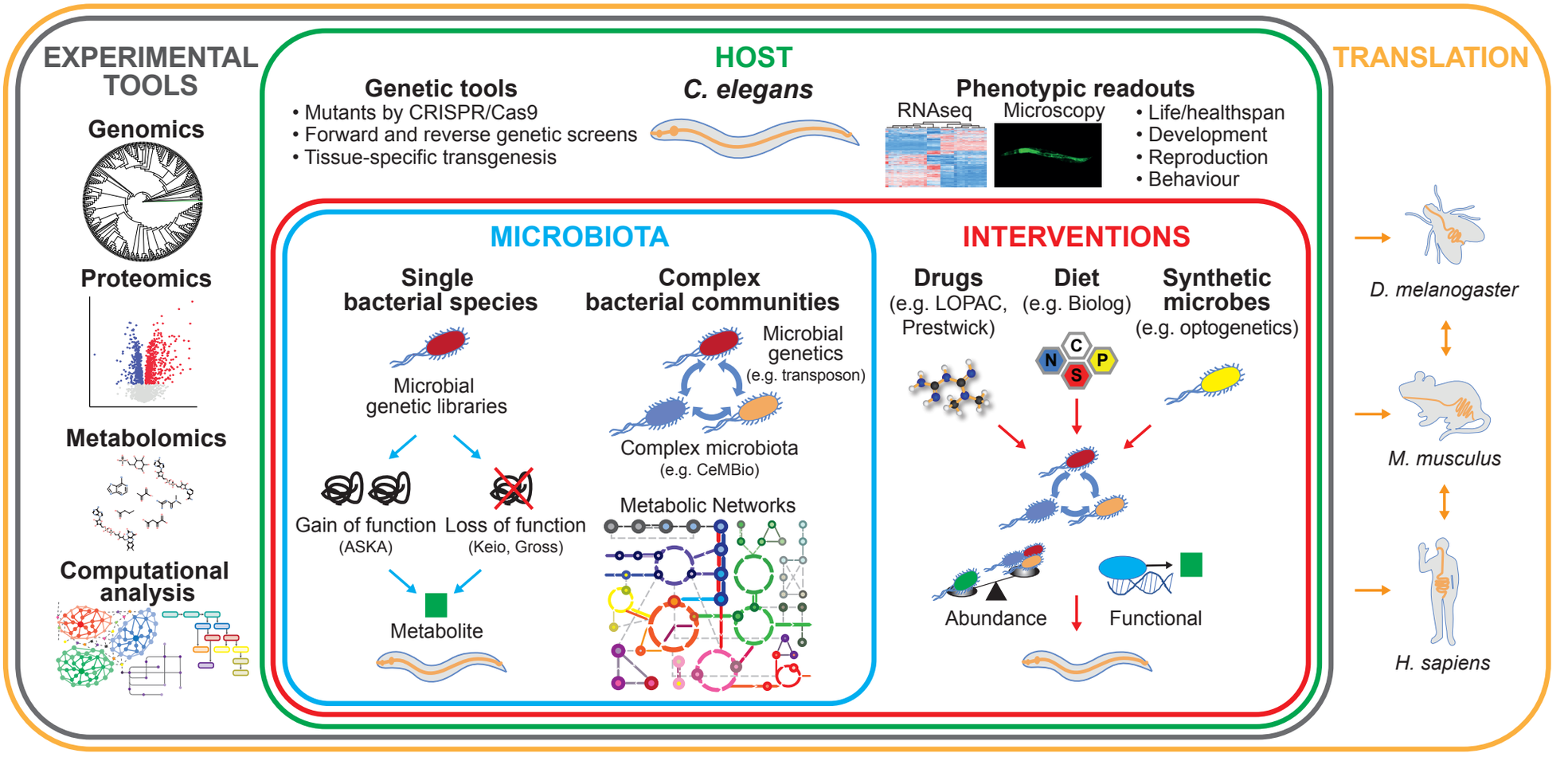
Serine regulates cancer drug effects through bacteria (Ke *et al.*[145])

Bacterial metabolites improve neurodegeneration (Goya *et al.*[64], Urrutia *et al.*[65])

Creation of mock microbiota CeMBio (Dirksen *et al.*[43])

Host-microbe freeloading and self-defense strategies (Schiffer et al.[53])

Bacterial small RNAs alter host behaviour (Kaletsky *et al.*[41])


EXPERIMENTAL TOOLS
Genomics
Proteomics
Metabolomics
Computational analysis
HOST
C. elegans
Genetic tools
• Mutants by CRISPR/Cas9
• Forward and reverse genetic screens
• Tissue-specific transgenesis
Phenotypic readouts
RNAseq
Microscopy
• Life/healthspan
• Development
• Reproduction
• Behaviour
MICROBIOTA
Single bacterial species
Microbial genetic libraries
Gain of function (ASKA)
Loss of function (Keio, Gross)
Metabolite
Complex bacterial communities
Microbial genetics (e.g. transposon)
Complex microbiota (e.g. CeMBio)
Metabolic Networks
INTERVENTIONS
Drugs (e.g. LOPAC, Prestwick)
Diet (e.g. Biolog)
C
N
P
S
Synthetic microbes (e.g. optogenetics)
Abundance
Functional
TRANSLATION
D. melanogaster
M. musculus
H. sapiens