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Teaser Understanding the complex phenotypes of aging and longevity requires careful examination and appropriate contextualization of multi-omic big data, strategic use of new technology, and a continued focus on animal models.

Moving beyond the current limits of data analysis in longevity and healthy lifespan studies

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Living longer with sustainable quality of life is becoming increasingly important in aging populations. Understanding associative biological mechanisms have proven daunting, because of multigenicity and population heterogeneity. Although Big Data and Artificial Intelligence (AI) could help, naïve adoption is ill advised. We hold the view that model organisms are better suited for big-data analytics but might lack relevance because they do not immediately reflect the human condition. Resolving this hurdle and bridging the human–model organism gap will require some finesse. This includes improving signal:noise ratios by appropriate contextualization of high-throughput data, establishing consistency across multiple high-throughput platforms, and adopting supporting technologies that provide useful *in silico* and *in vivo* validation strategies.

Introduction

The aging population is becoming predominant in many parts of the world, raising new challenges. Many industrialized countries are getting ready to deal with an impending 'silver tide', which could overload current healthcare systems and reduce overall economic productivity. Therefore, devising effective yet practicable approaches to help people live longer, good-quality, lives, and help extend economic productivity, is increasingly dominating conversations across many sectors, including the media, government policy-makers, scientists, and technopreneurs. This includes increased interest in new scientific, technological, and entrepreneurial innovations, leading to the establishment of a new 'longevity' industry [1].

Healthy aging and longevity are a function of natural biological change. Consequently, biotechnological and pharmaceutical research to develop novel drugs, healthcare products, and biotechnological implements will be the key drivers towards longer, good-quality, lives. Unfortunately, the underlying biological processes behind aging and longevity are multifactorial and are influenced by

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the interaction of both genetic and environmental factors. Most current knowledge is derived from model organisms. For instance, *Caenorhabditis elegans* research across the past decade positioned this organism at the forefront of the aging field. Several conserved signaling pathways with crucial roles in longevity have been identified in this microscopic worm. Glucose supplementation, calorie restriction, and fertility are paradigms considered relevant to human longevity [2]. Nonetheless, there is still a large knowledge gap, and model organisms can still surprise us. In a recent study, a high glucose diet was shown to slow down development and to reduce lifespan when provided from a young age while, surprisingly, extending longevity if administered post fertility [3]. This age-specific dissection is unexpected and would have been difficult to infer from human data. We have not yet learned all the essential contributing factors and conditions relevant to aging. Thus, this makes the continued deployment of model organisms both crucial and essential for advancing our understanding of aging in humans.

As biology becomes increasingly digitized, larger and more complex data sets emerge. Consequently, AI and machine learning on biological big data have become essential emerging technologies. Recently, the pharmaceutical giants Pfizer and Novartis collaborated with IBM to use Watson AI on big data to develop novel cancer therapies and improve health outcomes. New tech entrants are also emerging rapidly, such as XtalPi and its Intelligent Digital Drug Discovery and Development (ID4) platform, which integrates quantum mechanics, AI, and cloud computing, and allows pharmaceutical companies to increase their efficiency, accuracy, and success rates at crucial stages of therapeutic developments. Besides drug development, AI and big data are also used in cell imaging analysis (Allen Cell Explorer) and knowledge discovery (BenevolentAI). AI and big data are also pertinent for aging research (Calico). The inevitable reality is that biological research is increasingly digitized, and its future lies in the strategic maximization of data and use of new technologies.

Despite the new emphasis on AI and big data, animal models remain relevant. Undoubtedly, as de Magalhães elegantly phrases it, 'humans are not huge worms or big mice' [1]: current research paradigms tend to place higher value on clinical or human-based data, because they are perceived to be of direct biological relevance. Nevertheless, we feel that incredible potential remains and insights still gleanable from simpler organisms. For instance, a recent evaluation of global changes in protein turnover in aging *C. elegans* helped to implicate and characterize the phenomenon of proteome remodeling and aggregation with age [4]. Aging worms provided insights difficult to establish in human studies because of uncontrollable genetic and environmental variations.

It is narrow-minded to depend on AI and big data without considering its limitations. Where new cures and life-enhancing breakthroughs are concerned, we cannot understate the importance of human ingenuity [5]. Hence, there is a need to rein in the naïve optimism with a note of caution. Here, we highlight issues pertaining to modeling techniques, model organisms, and bioengineering techniques that we consider to be important in the context of aging and longevity research.

Model organisms should not be discounted: there are still incredible insights to be gleaned

Common model organisms are often portrayed as oversimplistic and difficult to generalize towards the human condition and have been criticized as being 'genetically homogeneous' within a 'controlled environment' [6]. However, changes observed over a uniform genetic background are often more meaningful and decipherable, especially while interrogating specific processes to dissect their role in aging and associated pathologies to inform on the human condition.

Aging is characterized by a gradual degeneration of physiological capacity and the diminished ability to cope with environmental stresses, which in turn leads to a heightened susceptibility for age-related diseases [7]. Several theories have been suggested to explain aging, including deregulated generation of reactive oxygen species (ROS), build-up of cell and tissue damage over time, accumulation of undegradable metabolic by-products that affect health, and altered physiological homeostasis [8]. The effects of ROS during aging are well studied: ROS induces cellular degeneration and senescence resulting from environmental exposure, therapeutics, and metabolism over the life of an organism (for an in-depth discussion, see [9]). Reactive compounds generated by these alterations can cause cellular damage, changing the properties of lipid membranes, cross-linking proteins, and inducing mutagenic changes to DNA. Among ROS-derived modifications, cytosolic protein carbonyls are major byproducts of oxidative stress [10]. With age, this balance is lost and ROS alongside protein carbonyls levels rise, leading to the accumulation of protein aggregates [11,12]. This process is not fully understood and its contribution to aging remains debatable.

Aging is also associated with alteration in organelles and their functions. In aging, mitochondria are perhaps the most studied, because of their involvement in ROS generation and metabolism. Mitochondria are known to both modulate and be altered by aging [13]. Mitochondrial stress has long been proposed to have a role in cellular aging. For instance, gradual reductions in mitochondrial metabolic enzymes and electron transport capacity, accompanied by increases in ROS production, are observed during aging [14]. Recent studies on mitohormesis, the cellular response to mild mitochondrial stress, have begun to unveil the intricate mitochondria–cytosol–nucleus crosstalk [15]. Early studies on RNA interference (RNAi)-mediated inhibition of electron transport chain and ATP synthase in *C. elegans* linked mitohormesis to longevity in worms [16]. The exact mechanisms of mitohormesis is not yet completely understood, but ROS, mitochondrial metabolites, stress response pathways, and mitokines have been implicated in the process [17]. The mitochondrial unfolded protein response (UPR^{mt}), first identified in mammalian cells [18], has also been shown to regulate longevity and healthspan in *C. elegans* [19], although the mechanisms involved might not be conserved between the two systems. In worms, where the process is better characterized, the mitochondrial exclusion of the ATFS-1 transcription factor during stress causes its translocation into the nucleus and activation of its target genes, which include mitochondrial chaperones, transporters, antioxidant enzymes, and cytoplasmic proteins that regulate mitochondrial fission and metabolism [20]. Studies on *C. elegans*, and later on flies, also showed the cell-nonautonomous nature of mitohormesis-induced longevity and the role of mitokines in the process [21,22]. Cytoplasmic stress response pathways contribute to mitohormesis. Mild mitochondrial stress activates the cytoplasmic heat stress response, leading to better cytoplasmic proteostasis and healthspan in worms [23]. Although UPR^{mt} has been suggested as a mechanism that mediates mitohormesis, several studies have shown that it is not sufficient to induce longevity alone [24], but might need other factors, including hypoxia-inducible factor (HIF)-1,

chromatin regulators, or HSF-1 [23,25–27]. Mitochondrial ROS has been shown to increase longevity in worms and might mediate mitohormesis, contrasting with increases in cytosolic ROS, which reduce lifespan [28]. Mitophagy is a quality-control system that regulates autophagic removal of damaged mitochondria and modulates cellular metabolic state. Mitochondrial damage that perturbs inner mitochondrial membrane transport causes PTEN-induced kinase 1 (PINK1) to phosphorylate ubiquitin attached to mitochondrial surface proteins, resulting in further ubiquitination by the E3 ligase parkin and recruitment of the damaged mitochondria to autophagosomes [29]. Furthermore, mitochondrial dynamics and function progress in a coordinated fashion, with mitochondrial fusion associated with increased ATP production and its inhibition associated with reduced oxidative phosphorylation and increased ROS production [30]. Disruption of mitochondrial dynamics affected longevity, and muscle and neuronal function in *C. elegans*, with more drastic effects upon inhibition of mitochondrial fusion than of fission [31]. These and many other studies, which are outside the scope of this review, suggest that mitochondrial functions are major nodes in longevity-associated networks and that the gradual deterioration of mitochondrial health during aging is a potential target for intervention in aging studies.

A gradual reduction in cellular quality-control systems is a characteristic of aging that also affects other organelles. Oxidative and endoplasmic reticulum (ER) stress also intersect because some forms of ROS can activate the ER stress response, referred as the UPR [32,33]. To counteract aging, the UPR mitigates cellular damage brought about by stress conditions [34,35], although the UPR transcriptional responsiveness tends to decrease with age [36,37]. A similar decline has been observed for other homeostatic cellular stress responses (reviewed in [38,39]). Together, these interconnected stress responses are influenced by environmental exposure, lifestyle, and disease, making it challenging to confidently correlate these phenomena with aging in humans.

Mice are the most used mammalian aging model. Their small size and relatively short lifespan are advantageous. The development of amenable genetic manipulation systems have made them the best system to interrogate the effects of genes and environment on mammalian aging. Alterations in several cellular processes, such as DNA damage repair, loss of proteostasis, epigenetic alterations, and metabolism, have led to distinct mouse models [40]. Given that studying aging in mice is lengthy and laborious, model organisms with shorter lifespan that are easily manipulated have been instrumental to dissect the molecular mechanisms that modulate aging. In *C. elegans*, the insulin/insulin growth factor (IGF)-1 signaling (IIS), target of rapamycin (TOR) signaling, and germline signaling pathways, which modulate development, reproduction, metabolism, somatic maintenance, and stress resistance, are associated with longevity (Fig. 1). For instance, mutations in the phosphatidylinositol 3-kinase gene *age1* or insulin/IGF-1 receptor *daf2* gene double the lifespan of *C. elegans* [41,42]. Reduced IIS activity lifts the

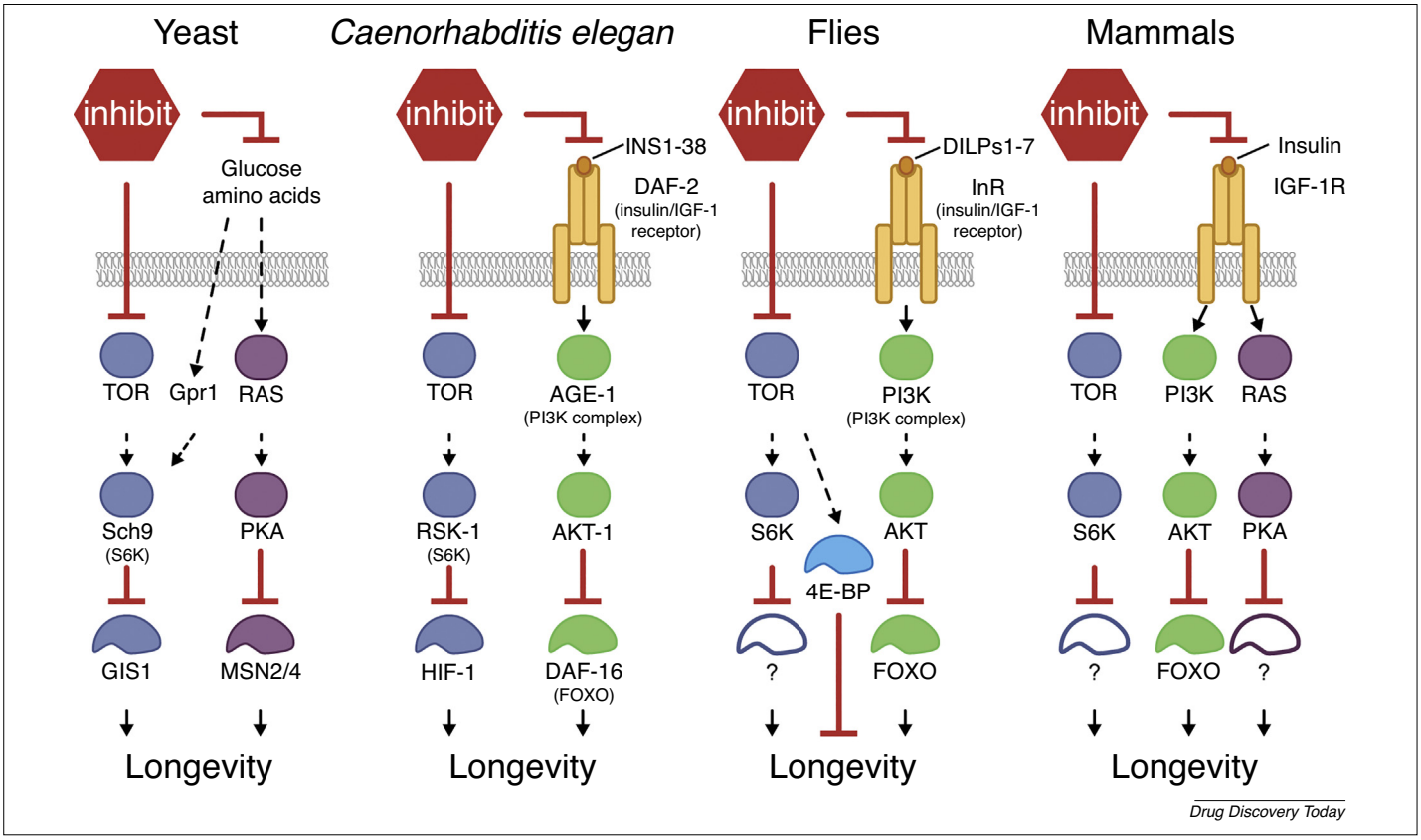

FIGURE 1

Insulin and target of rapamycin (TOR) signaling pathways are highly conserved and promote longevity. The activity of the insulin and TOR signaling pathways is partially inhibited by dietary restriction, promoting longer lifespan. Similarly, drug-induced inhibition or deletion of any proteins upstream of the transcription factors [GIS1, MSN2/4, hypoxia induced gene 1 (HIG-1), DAF-16, Forkhead box O (FOXO), and 4E-BP] result in higher activity of their respective downstream transcription factors, promoting longevity. By contrast, higher activation of the insulin and TOR signaling pathways in response to a high calorie diet or genetic manipulation reduces lifespan.

inhibition of the downstream Forkhead box O (FOXO) transcription factor DAF-16, inducing the expression of genes that increase lifespan (reviewed in [2,43]). By contrast, the lack of functional DAF-16 dramatically reduces *C. elegans* lifespan. IIS regulation of longevity is conserved, with similar long-lived IIS mutants found in *Drosophila melanogaster* [44,45] and mice [46,47]. Nonsynonymous polymorphisms in the gene encoding the IGF-1 receptor, associate with longevity have been identified in cohorts of Ashkenazi Jewish centenarians, suggesting that IIS attenuation also extends human lifespan [48]. However, long-lived IIS mutations might come at the cost of reducing body size, because small dog breeds live longer compared with larger dogs possibly because of distinct polymorphisms in *Igf1* [49,50].

In the search for methods to extend longevity, drugs targeting TOR signaling have been developed and tested in animal models and clinical trials. Rapamycin is probably the most commonly used drug to inhibit TOR, which promotes longevity in mice even if fed late in life [51]. Several drugs have been tested in clinical trials to treat Hutchinson–Gilford Progeria syndrome, which is a sporadic, autosomal dominant disorder characterized by premature and accelerated aging [52]. In this regard, Human Ageing Genomic Resources (HAGR) has collected information on >300 genes that are known to affect human aging and >2000 genes that are known to affect model organisms. It also lists >400 drugs that are known to affect aging [53]. The National institute of Aging supports the Intervention Testing Program to identify nongenetic treatments that can be used to modulate aging [54].

As *in vitro* models of aging, replicatively senescent cells have been instrumental in characterizing biochemical, signaling, genetic, and epigenetic changes associated with aging [55]. Replicative senescence is affected by the attrition of telomeres during cell division. Although the use of this model system has implicated telomeres in human aging, this process is absent or dissimilar in *C. elegans* and mice [56,57]. However, several aging-related pathologies that are closely related to telomere biology suggest a strong link between human aging and telomeres [58]. Senescence has been described as a defense against uncontrolled cell division and cancer, but recent studies have shown that the relationship between these processes is neither simple nor linear and *in vitro* senescence-associated processes are not always associated with organismal aging, but are also found as a part of the celluar stress response mechanism. Thus, care has to be exercised while extrapolating observations from cell culture-based senescence studies to aging in humans or model organisms. Stress-induced senescence is often intimately related to the local inflammatory processes and is a driving factor in cancer cell survival and growth. Recent reports implicated the senescent cell secretome, which include inflammatory signals, in cancers and chronic age-associated pathologies [59]. Senescence-associated secretory processes are regulated by signaling pathways, including pleiotropic transcription factor nuclear factor (NF)-κB and mammalian target of rapamycin (mTOR) [60,61]. Such observations suggest that senescent cells have a pathological role in aging-associated disorders and could be therapeutic targets. The ablation of negative regulators of the proliferation protein $p16^{Ink4a}$ in a BubR1 progeroid mouse background showed that removal of senescent cells was beneficial to healthy aging [62], which has been validated in other studies [63,64]. This led to the development of senolytic drugs that selectively remove senescent cells in tissues [65]. Recently a two-center clinical trial of dasatinib together with quercetin to treat idiopathic pulmonary fibrosis suggested potential beneficial effects of senolytics in age-associated disorders [66].

Adding to the complexity of genetic variations, diet greatly modulates longevity in animals, and this is mediated. at least partly. in an IIS-dependent manner (Fig. 1). Calorie excess reduces longevity whereas chronic dietary restriction extends lifespan in several animal models. Calorie excess in the form of a high glucose diet reduced the lifespan of *C. elegans* [67], *Drosophila* [68], and mice [69]. By contrast, calorie restriction without malnutrition was first shown to increase lifespan in rodents and later in other model organisms, including *C. elegans* [70–72]. Three studies on the effects of calorie restriction on life health and survival of rhesus monkeys (*Macaca mulatta*) are apt examples of the relevance and restrictions of model organisms in preclinical aging studies. The first study showed a 2.6-fold increase in the risk of death in 109 *ad libitum*-fed control animals compared with 12 calorie-restricted male rhesus monkeys [73]. However, the two subsequent studies reported conflicting results on the effects of calorie restriction on aging: The University of Washington study showed clear benefits of calorie restriction on aging and health [74,75], whereas the other, carried out at the National Institute on Aging, showed almost no benefit conferred on aging with minor contribution to health [76]. Further analysis of these studies showed that the differences could be attributed to diet variability between animals used in the studies and revealed the existence of a minimum calorie restriction level that confers maximum benefits in nonhuman primates [77]. This analysis further showed the role of body weight (which might reflect basal nutrition levels) in realizing benefits from calorie restriction. Additionally, the analysis highlighted major gains of calorie restriction in adult or advance-aged rhesus monkeys, whereas the benefits in younger animals were shown to depend on sex and other factors. Together, these studies show that, although complex biological processes are more easily identified in simpler model organisms, higher and phylogenetically closer systems to humans will help us recognize finer but crucial details modulating aging. Consequently, a wave of calorie restriction and fasting diets emerged in the general population despite the lack of evidence linking diets and longevity in human. Recent randomized clinical trials on the effect of a 2-year calorie restriction failed to associate a decrease in serum IGF-1 concentration to calorie restriction as found in rodents [78,79]. Evidently, IIS and calorie restriction are part of a complex equation that extends youthfulness and lifespan in human. Although several conserved signaling pathways have crucial roles in longevity, we have not yet learned all the essential contributing factors and conditions relevant to aging. This makes the continued deployment of model organisms both crucial and essential to accelerate and solidify our understanding of aging in humans.

To overcome the limitations of aging studies, the integration of 'omics technologies with model organism studies are more likely to yield success because of a cleaner background and the ability to better control confounding factors. Recent reports showcase clear-cut advantages in deploying big data analytics on samples with cleaner backgrounds. These include the proteomic profiling of worms through stages of aging [37], tissue-specific proteome characterization of aging mice [80–83], tissue-specific transcriptome profiling of aged

C. elegans [84], astrocyte-specific transcriptome profiling of aging mouse brain [85], and the comparison of tissue-specific transcriptomes between aging animal models and humans [86].

Network analytics for cross-species comparisons: revisiting network alignment algorithms

Multidimensional high-throughput analysis of data is problematic because of confounders [87], noise, missing data, and collinearity effects. These lead towards direct violation of the independent and identically distributed (IID) principles often assumed in statistical testing. That is all samples have an equal chance of being selected, and the selection of one sample does not influence the selection of others. Issues, such as batch effects, are endemic and persistent, such that nondetection does not imply absence [88,89]. These issues must be addressed early to avoid overfitting and nongeneralizability where any model developed is overly attuned to the idiosyncrasies of the data set itself and has no biological meaning, such that the model will fail when tested against another independently derived data set [90–92]. The sophistication of current AI is not a guaranteed hedge against poor-quality data.

High-throughput biological data are better expressed as networks. Biological networks refer to a heterogeneous assortment of models, including protein–protein interactions, and metabolic, biochemical, regulatory, and signaling pathways (for an in-depth discussion, see [93,94]). As an analogy, street names are unusable for navigation if presented as a list. How the roads are arranged against each other to move from point A to B is more important. Similarly, biological molecules bind and work with each other, forming modules, clusters, and complexes, creating a collective network system. For example, the biological process of glycolysis involves ten proteins that transforms glucose into pyruvate. The names and identities of the proteins and by-products are not useful as an unordered list. It is also difficult to think about the process as 3D molecules and real-time processes. Instead, the process can be abstracted into a bipartite network system with two classes of node: the proteins and the metabolites, with a directed connection linking each node so that precedence and directionality can be established. In fact, this form of simplified representation can be found in any biochemistry textbook (although not thought of formally as networks). Therefore, viewing biological processes not as lists, but as networks is more natural and informative. Indeed, applying statistical analysis not to individual genes but in terms of their constituent networks and pathways improves the signal: noise ratio, reduces collinearity issues, improves resistance against technical bias and batch effects, and provides resistance against missing data [95].

To identify conserved components between related organisms in aging, a specific class of network algorithms is required, network-alignment algorithms (NAAs). NAAs create the best topological fit between two networks of the same or different species. Within the same species, NAAs identify local rewiring events because of drug response or disease. These, in turn, reveal the underlying mechanisms [95]. For different species, NAAs are valuable to understand evolutionarily conserved and divergent events. However, this requires making more assumptions, such as establishing interspecies gene homology. Fortunately, tools such as Ortho-Finder tool have been developed to address this problem [96].

As with sequence alignment algorithms, many NAAs work by comparing pairs of networks. NAAs maximize either local or global similarity. Local network alignment (LNA) algorithms search for small parts of the networks that are similar and possibly represent conserved functional structures (Table 1). Alternatively, global network alignment (GNA) algorithms identify the best overall alignment between two entire networks. Recently, it was reported that LNAs are suitable to recover functional information but do poorly in topological alignment quality, whereas the opposite is true for GNAs [97].

Some NAAs depend purely on the pattern of connections in the network, referred as topology, whereas others incorporate biological contextual clues, such as sequence similarity. Although it is tempting to simply select methods that use biological information, these usually only produce good alignments between networks of closely related species. That is, methods that produce alignments using biological information that suggests high similarities will work well when you already expect them to [98]. Among NAAs utilizing biological information, the performance evaluation of Clark and Kalita [99] suggests that NAAs, such as SPINAL [100], NATALIE [101], and PINALOG [102], are useful because they produce quick alignments with competitive results. Among NAAs that utilize only topological information, AlignMCL works well and is relatively accessible because of Cytoscape plugin implementation [103]. Other NAAs, such as GRAAL [104], leverage on heuristics to speed up computation, using interesting generalization and pattern capture techniques, including Graphlets (Table 1).

The study of aging in humans is difficult experimentally because of the long human lifespan and ethical constraints [105]. Additionally, intergroup comparisons might easily lead towards chance associations because of human heterogeneity [87], whereas animal models produce reliable data. However, the knowledge generated using these animal models needs to be transferred computationally to support the human condition, and this can be achieved using NAAs.

Given that aging and longevity are not human specific, we can intersect common aging-related components across species. Given a set of longevity-related networks, conserved components could reveal more about aging. This requires two tasks that include networks: (i) resolving the missing entity problem; and (ii) identifying conserved elements across species that age.

The missing entity problem refers to the inconsistent observation of biological molecules correlating to a phenotype. It is similar to the missing protein problem (MPP), where incomplete data causes knowledge 'gaps' [106,107]. Resolving the missing entity problem requires 'guilt-by-association' types of analysis, which involves identifying a common link among genes of unknown characteristic associated with genes of known characteristic. For example, given a list of known cancer genes, novel cancer genes might be identified in a biological network by identifying other genes highly connected to the input list of cancer genes [108]. Similarly, the statistical enrichment for cancer genes found within complexes and pathways can be used to associate unknown or unreported genes within these same complexes and pathways [109,110].

TABLE 1

Examples of network alignment algorithms used in biological research

Algorithm	Local	Global	Pairwise	Multiple	Functional	Topological	Refs
pathBLAST	Yes		Yes		Yes	Yes	[153]
NetworkBLAST	Yes		Yes		Yes	Yes	[154]
MaWISH	Yes	Yes				Yes	[155]
IGLOO	Yes	Yes	Yes		Yes	Yes	[156]
AlignNemo	Yes		Yes		Yes	Yes	[157]
ConvexAlign		Yes		Yes	Yes	Yes	[158]
FASTAn		Yes	Yes		Yes	Yes	[159]
HubAlign		Yes	Yes		Yes	Yes	[160]
AlignMCL	Yes		Yes			Yes	[161]
GEDEVO		Yes			Yes	Yes	[162]
MAGNA++		Yes				Yes	[163]
WAVE		Yes				Yes	[164]
multiMAGNA++		Yes		Yes	Yes	Yes	[165]
FUSE				Yes	Yes	Yes	[166]
ModuleAlign		Yes	Yes		Yes	Yes	[167]
GRAAL		Yes	Yes			Yes	[168]
H-GRAAL		Yes				Yes	[169]
MI-GRAAL		Yes			Yes	Yes	[104]
C-GRAAL		Yes	Yes			Yes	[170]
L-GRAAL		Yes				Yes	[171]
IsoRank		Yes	Yes	Yes	Yes	Yes	[172]
NSD		Yes				Yes	[173]
IsoRankN		Yes		Yes	Yes	Yes	[174]
Greamlin		Yes		Yes	Yes	Yes	[175]
SMETANA		Yes		Yes	Yes	Yes	[176]
NetCoffee		Yes		Yes	Yes	Yes	[177]
BEAMS		Yes		Yes	Yes	Yes	[178]
PISwap		Yes	Yes		Yes	Yes	[179]
MAGNA		Yes	Yes		Yes	Yes	[180]
Optnetalign		Yes		Yes	Yes	Yes	[181]
GHOST		Yes	Yes		Yes	Yes	[98]
NATALIE		Yes	Yes		Yes	Yes	[182]
NETAL		Yes	Yes		Yes	Yes	[183]
SPINAL		Yes	Yes		Yes	Yes	[100]
PINALOG		Yes	Yes		Yes	Yes	[184]

To explore the conservation and divergence of signaling pathways, functional modules, and regulatory relationships, LNAs can be used by comparing interspecies biological networks [111]. The process requires mapping the nodes of one network to the nodes of another, such that the mapped nodes correspond to both their place in the network topology and their biological attributes. By this approach, undiscovered homologies among different species and functionally similar subnetworks can be identified [99]. Similarly, by systematically comparing evolutionarily divergent species, conserved components of aging pathways can be revealed.

Pathway conservation can be studied experimentally in a low-throughput manner using LNAs [112]. For example, rather than aligning the entire network using GNAs, we can use prior knowledge on a gene subset or a pathway, and parsimoniously eliminate irrelevant components using LNAs. Moreover, once the networks are aligned, further independent proof is required before experimental validation can be carried out. *In silico* approaches are cost-efficient here. For instance, the bioinformatics tool PANTHER [113] can identify domain conservation. Alternatively, NAAs can be directly integrated with hidden Markov models (HMM), which naturally integrate both 'node similarity', estimated by sequence similarity, and 'interaction reliability' into the scoring scheme for comparing aligned paths [114,115]. Given that different species might not have the exact same pathway components, HMMs can identify indel events, where new pathway components can be added or lost throughout the course of evolution (Fig. 2). In turn, aligning networks can explain similarities and differences in biochemical processes or phenotypes, which is ultimately inferential and produces models difficult to verify. However, synthetic biology has recently taken the approach of re-engineering pathways and networks to validate predictions *in vivo*.

Humanizing model organisms via synthetic biology

Synthetic biology has been instrumental to biochemically engineer new pathways or alter existing ones to better understand how individual pathway components work. Advancement of gene-editing technologies, such as CRISPR/*Cas9*, and others, have made it possible to carry out large-scale pathway re-engineering of multiple gene sets. Deploying NAAs is potentially a preliminary guide to identify genes to modify, insert, or remove, in the bid to humanize model organisms and obtaining relevant insights into the human condition.

The need to industrially synthesize compounds has significantly contributed to the growth of synthetic biology. For instance, *S. cerevisiae* was engineered to produce the bright-red carotenoid bacterial lycopene with high purity and stability [116]. Lycopene is

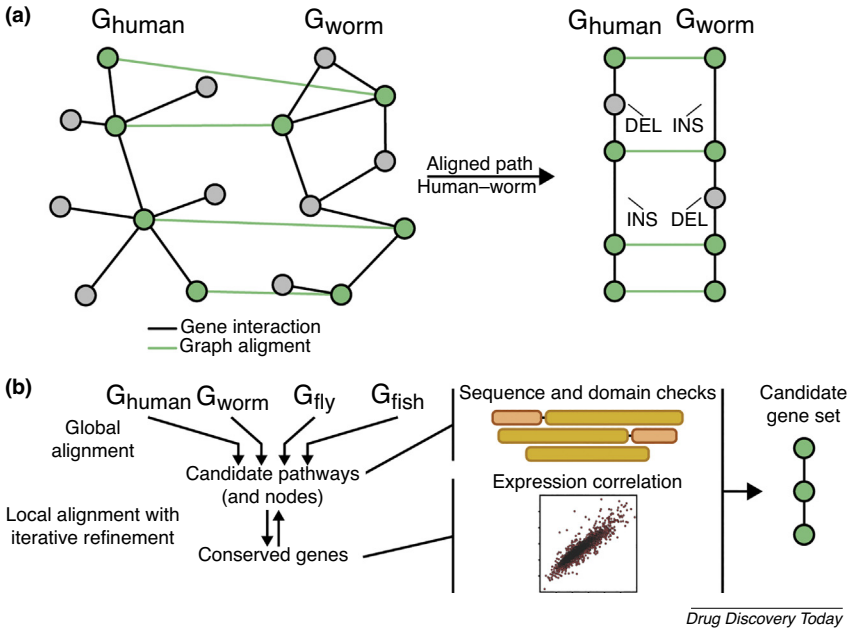

FIGURE 2

How network alignment can be used to identify minimal conserved components between species. (a) Network alignment between two toy networks (GHuman and GWorm). Network alignment approaches that consider both topology and sequence similarity coupled with 'machine-learning capabilities' are robust and can incorporate insertion-deletion events (shown in gray). In practice, we can use alignments for identifying conserved components among species that exhibit 'aging' phenotypes. (b) The process of a multispecies comparison (human, worm, fly, and fish) can help identify conserved pathways and networks. Genes belonging to these conserved pathways and networks can be verified for functional coherence (via sequence and functional domain checks) and checking for conserved expressional correlations to improve the confidence that they act as a functional unit. Same-pathway genes that pass these checks are considered as candidate genes belonging to a conserved pathway.

used industrially as a natural colorant and food additive. The same research group designed a synthetic route to achieve odd-numbered chain fatty alcohols [117] as well as synthesizing different compounds by improving the adaptability of the exogenous modules and the endogenous pathways, including aliphatic hydrocarbons [118], salvianic acid A [119], geraniol [120], and crocetin [121]. Yeast has also been exploited to potentially manufacture drugs including noscapine [122,123] and opiates [124]. Therefore, the interdisciplinary field of synthetic biology has flourished into the development of complex systems with potential applications to answer fundamental questions of biological functions.

An international interinstitutional team is making efforts to build the first synthetic eukaryotic genome, which offers the possibility to interrogate isolated pathways in a controlled setting [125]. For instance, synthetic and scrambled yeast chromosomes lacking repeats are used to study 3D genomic organization, providing clues to genetic regulations [126]. Similarly, the replacement of >400 essential yeast genes with human orthologs proved to be a suitable approach to demonstrate the similarity of these evolutionary distant species [127].

With a common ancestor, human and yeast share thousands of orthologous genes that retain their original function over 1 billion years of evolution. Consequently, efforts have been made to successfully demonstrate that approximately half of the yeast genome can be replaced by corresponding human gene orthologs, including shared orthologs that are essential in both species [127]. In humans, some of these transferable genes are associated with disease, including cancer. Despite being a simple organism, yeast is a versatile and relevant platform to elucidate biological functions of human genes, proteins, metabolic pathways, and mechanisms relevant to human diseases.

Efforts have been made to engineer complex human pathways in yeast. As such, the yeast *Pichia pastoris* was humanized to secrete human glycoproteins with fully complex terminally sialylated N-glycans [128]. Yeast and other lower eukaryotes lack the ability to add terminal sialic acids to glycoproteins. This was a crucial tool to investigate protein modification functionality. This synthetic biology approach led to the publication of a detailed method to secrete human glycoproteins in *P. pastoris* [129] and the creation of biotech firm GlycoFi Inc., as a subsidiary of Merck & Co., to develop biotherapeutics based on the glycan optimization technology. Therefore, constructing complex pathways with multiple genes provides an opportunity to understand the entire functional context of a biological activity, which can lead to translational applications. However, the major limitation to this might be incomplete information, because not all members of a pathway of interest are known and corresponding endogenous yeast pathways must be completely disabled. This can be solved by synthetic genomics, where the predicted network can be tested in a foreign host.

A complex system has been developed to generate synthetic chromosomes capable of being modified within the host, referred as the Synthetic Chromosome Rearrangement and Modification by LoxP-Mediated Evolution (SCRaMbLE) system (Fig. 3). This designer synthetic yeast chromosome 2.0 (Sc2.0) encodes hundreds of loxPsym sites positioned downstream of nonessential genes and other major landmarks [130]. Sc2.0 is exclusively sensitive to the expression of Cre recombinase, a tyrosine recombinase enzyme derived from the P1 bacteriophage, which catalyzes

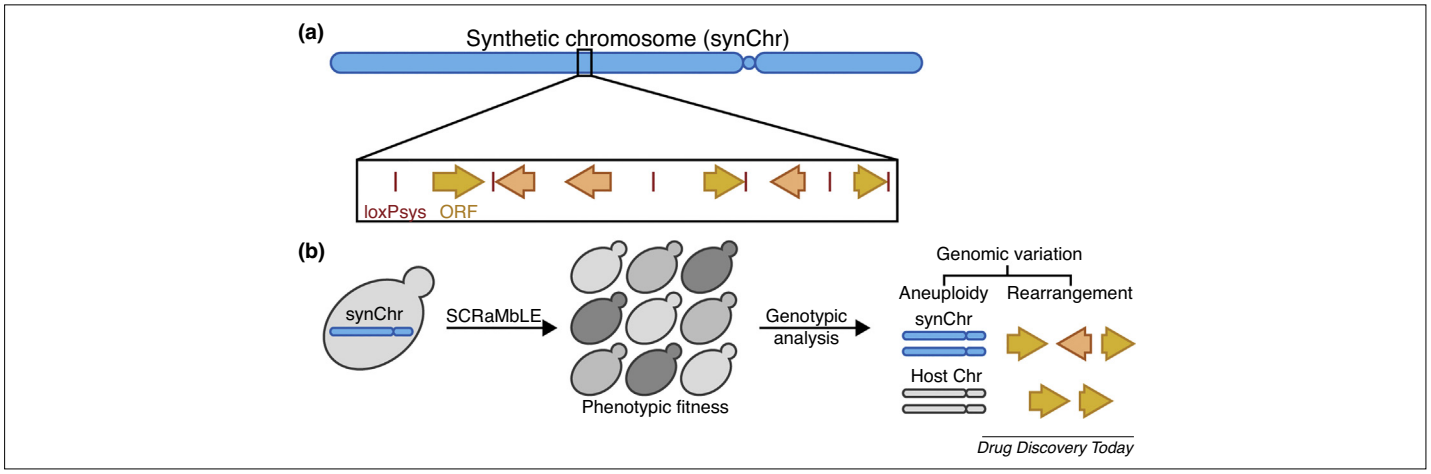

FIGURE 3

The Synthetic Chromosome Rearrangement and Modification by LoxP-Mediated Evolution (SCRaMbLE) system promotes genotype diversity, including deletions, inversions, duplications, and other complex rearrangements. (a) The SCRaMbLE system can contain open reading frames (ORFs) from yeast and/or other species. Genes are inserted through loxP site systems (loxPsys). (b) Yeast strain containing a synthetic chromosome (synChr) is grown under stress and/or selective conditions, resulting in diverse rearrangement of the synChr by SCRaMbLE. The subsequent yeast strains will have different phenotypic fitness. Sequencing of these strains reveals genetic variation, including aneuploidy of the synChr or the host chromosome (host Chr) and genetic rearrangement of the synChr.

site-specific recombination of DNA between loxP sites. Previous studies demonstrated that the synthetic yeast chromosome SCRaMbLE system promotes genotype diversity, including deletions, inversions, duplications, and other complex rearrangements [130–132]. Thus far, the synthetic yeast chromosomes synII, synIII, synV, synVI, synIXR, synX, and synXII have been fully synthesized and incorporated into *S. cerevisiae* without major fitness defects [126,130,131,133–135], offering an opportunity to generate tremendously diverse host yeast strains that can be screened for the production of high added-value biomolecules [136]. To improve the orthogonality of SCRaMbLE, a DNA element rearrangement was developed on the basis of a pairwise orthogonal recombination system comprising the two site-specific recombinases, Vika/vox and Cre/loxP, in *S. cerevisiae* [137]. Combined with integrative analysis of high-throughput data coupled with network-based analysis and augmented with AI-led predictions, it is not unfathomable that one can predict genes associated with aging-relevant complex biological pathways and networks that can be tested with the SCRaMbLE platform. This approach would help to identify genes that are essential to the pathway or network and those that are dispensable.

Eventually, these tools could be exploited to assess the role of specific human pathways or subset of human genes in modulating aging. However, the risk remains that contributing genes might be inadvertently left behind whereas others might produce partial functional or mislocalized proteins in the host, altering the conclusions.

'Integrative multi-omics' and its role in providing more holistic insights

The integration of various high-throughput platforms for the purpose of providing high-powered data analysis is a key advantage attributable to multi-omics. The 'omics platforms are not independent of each other but are mutually supportive (Fig. 4a).

For example, the central dogma of biology states that genes are transcribed into mRNA and then translated into proteins. Therefore, the respective high-throughput technologies for assaying mRNA and proteins, transcriptomics, and proteomics should provide some measure of correlation such that a gene that is consistently high in both platforms is a corroborated result, whereas another that is not agreed on by either, presents an anomaly that could either be a false positive or could be explained by alternate mechanisms. Multi-omics can reveal the complexity of biology, whereas interplatform corroboration provides some protection against false positives and even false negatives. Integrated transcriptome and proteome analysis of aging rats along with ribosome profiling and phosphoproteomics revealed that changes in translation correlate with altered protein abundance [138]. The authors compared four data sets from the livers and brains of young and old rats to derive age-related changes, including altered translation, protein abundance, protein localization, alternative splicing, and altered protein phosphorylation. Such a multi-omics approach is costly and laborious but provides a dynamic overview of age-related changes that is otherwise unobservable on any single platform. Therefore, a strategic approach towards data management by taking into consideration the strengths and the potential insight expected from each platform (Table 2), as opposed to pure AI data-dumps, is a more reasonable strategy that is worth advocating.

In multi-omics analyses, contextualization based on biological networks already serves as a powerful unifier across the various high-throughput platforms. By searching for localized and consistently affected pathways or subnetworks, we considerably reduce the search space for causal factors [139]. To reduce the curse of dimensionality, the network perspective is useful for reducing false positives and false negatives [95]. This contextualization paradigm works because biological entities work naturally as systems, networks, and pathways. A protein complex is a functional unit comprising the biological entities of proteins and can be regarded as a generalization of a subnetwork; that is, a smaller network that forms part of a bigger network system. It is routine to predict protein

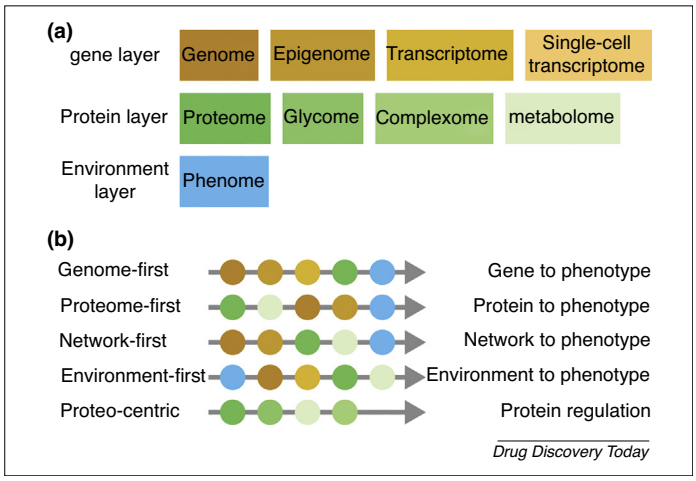

FIGURE 4

A simplified overview of multi-omics. Organizational hierarchy of multi-omics platforms (a) and various multi-omic integration strategies (b). Note that the color coding of 'omics technologies defined in (a) applies to (b). In (a), as an arbitrary division, we can broadly divide current high-throughput ('omics) platforms based on the type of molecules being assayed. For example, gene layers pertain to nucleic acid-based molecules, such as DNA, and also widespread chemical modifications on DNA, the aptly named epigenomics. The protein layer, although used in some multi-omics literature, comprises a more heterogeneous mixture of molecules, and does not comprise proteins alone, covering metabolic products that might be by-products of enzymatic reactions (the metabolome). This layer also includes chemical modifications of proteins, such as glycosylation changes (the 'glycome'). A higher-level abstraction is the organization of proteins into protein complexes, known as the complexome. It is now recognized that the phenotypic information, including that of a clinical nature, can be complex and highly multivariate (phenome). In (b), various 'omics platforms can be chained together to produce 'multi-omics' pipelines. Shown here are some common chaining strategies.

complexes from protein–protein interaction networks [140]. It is also reported that protein complexes are more biologically coherent than most sources of biological information [141]. Hence, studying biological networks directly is advantageous. Given advancements in modern mass spectrometry and chromatographic techniques, we are now able to assay subnetworks as complexes in a high-throughput manner. In a recent profiling of the HEK293 cell line proteome, 462 complexes comprising 2127 protein subunits were traced in a single operation [142]. Protein complexes and aging are linked because a loss of stoichiometry in certain protein complexes [e.g., nuclear pore complex (NPC)] occurs with aging [143]. Therefore, this emerging 'omics paradigm cannot be ignored. We expect that a complexomic-based profiling of aging will be a powerful technique to use to develop a network view of aging.

In practice, limitations of multi-omics include cost, availability of technology and expertise, and sample availability. Therefore, the aim must be to anticipate the best multi-omics route to navigate (Fig. 4b). Genomics and transcriptomics are mature technologies that support large sample sizes with low technical errors, but are distant to biological functions and phenotypes. Conversely, a proteome-first perspective provides direct insight into functionality, but is hampered by consistency and coverage issues. Furthermore, direct integration between the genome and proteome is often difficult because of poor correlations [144]. Poor correlation between 'omics platforms might not always suggest additional regulatory layers, but can simply be a consequence of technical noise, batch effects, and inaccurate measurements.

Statistically, simple correlation and context mapping are the two major approaches for integration [145]. A simple correlation considers whether pairs of biological entities behave similarly, such as co-expression in similar tissues or change in terms of their expression levels in a concerted manner. Simple quantitative assessments to establish correlations between 'omics platforms include the often-deployed Pearson product moment coefficient (PPMC) and the Spearman rank-order correlation (SROC). Both PPMC and SROC measure the extent to which two variables change together. The primary difference between PPMC and SROC is that the former evaluates the linear relationship between two continuous variables, taking into account the actual values, whereas in SROC, only the monotonic rank-based ordering is considered. By contrast, context mapping assesses whether differential sets of genes and proteins co-map to similar pathways and subnetworks, or if there are enrichments for common or cohesive functional terms, cellular locations, or disease.

In practice, the efficacy of context mapping depends on many factors, such as the completeness of the reference network or pathway database. *C. elegans* has the reliability in terms of false positives and false negatives of the 'omics platform. Nonetheless, simple yet imperfect context-mapping techniques have been sufficient to inform on the aging condition [146]. Enrichment analyses have implicated biological processes, such as immune response, lysosome, glycoproteins, and mitochondrial activity, to longevity [147]. The greatest limitation in context mapping might be the lack of quality reference network and pathway databases and appropriate analytical techniques [148]. The incomplete pathway database problem can be overcome by estimating those components that should be present based on integrating cell- and condition-specific high-dimensional 'omics data with interacting cues from existing databases [149]. Ultimately, multi-omics integration techniques provide a limited snapshot of complex and dynamic biological processes.

Given these unresolved issues in data analysis, advance achine learning, AI, and big data are tempting tools to use to overcome these obstacles. It appears sensible to take advantage of this new technological wave. The phenome-first Aging.AI system is a deep learning AI tool that can identify aging biomarkers to develop antiaging therapies [150]. This platform addressed the issue that ethnic differences might be a strong confounder over other variables of interest, such as diet, behavior, and lifestyle. Consequently, confounders of variables of interest overshadowed potential ethnicity confounders across a large combined data set of Canadian, South Korea, and Eastern European populations [150]. Similarly, the AI platform PhotoAgeClock was developed as a deep learning algorithm for non-invasive visual biomarkers of aging [151]. PhotoAgeClock was developed by comparing thousands of individual eye corner images over a chronological range of 20–80 years. However, these AI-led approaches failed to address multi-omics integration issues directly. Machine learning, AI, and any other analytical techniques are also constrained by data heterogeneity and quality [152]. Therefore, robust approaches are urgently needed to accurately develop models based on multi-layered multi-omics data and to facilitate data integration. These future

TABLE 2

A descriptive summary of high-throughput ('omics) platforms currently used in biological research

Platform	Description	Limitations	Strengths
Genomics	Profiles a sample genome at the genomic DNA sequence level; provides insights into single-nucleotide and gene copy number changes	Gene sequence change does not translate directly into phenotype	Matured technology for both hardware and analytics; relatively inexpensive and accessible
Epigenomics	Profiles genome-level biochemical changes that do not include DNA sequence mutations; biochemical changes include methylation and acetylation	Limited genome-level coverage; many identified changes have no functional impact	Insights into environmental and lifestyle factors
Transcriptomics	Profiles total RNA changes at the ribonucleotide level based on RNA sequence; provides insights into actual gene expression and genetic constitution of an organism	RNA sequence change does not translate directly into phenotype	Matured technology for both hardware and analytics; relatively inexpensive and accessible
Proteomics	Profiles total protein-level changesl; highly flexible platform; provides insight into protein expression levels, but can also be retooled for monitoring peptide- and sequence-level changes	Expensive and difficult to scale; coverage and consistency issues	Directly assays proteins that relate to observed phenotype
Glycomics	Profiles high-throughput structural analysis of protein glycan proteome-level biochemical changes	Many other biochemical modifications exist	Helps to explain modulation in protein functions because of glycosylation
Metabonomics	Profiles low-molecular-weight molecules, such as biochemical by-products, in a biological system; there are >40 000 distinct metabolites; subcategories include lipidomics, which is dedicated to the measurement of lipids in the sample	Difficult to assay and profile all metabolites	Expands biomarker set beyond gene and proteins; provides evidence for activity of genetically controlled pathways
Phenomics	Profiles observations and metrics (phenotypes) in the organism; also includes high-dimensional clinical data and other associated comorbidities	Requires extensive analysis and collection of data	Insights into interdependencies and correlations among various phenotypes
Single-cell transcriptomics	Similar to transcriptomics, but captures information at level of individual cells	Expensive and difficult to scale up; poorer quality data that are difficult to analyze	Provides ability to assay cellular subpopulations and lineage tracing
Complexonomics	Profiles the set and make-up of protein complexes expressed in samples	Expensive and difficult to scale up; coverage and consistency issues	Although proteins account for functions, they work in units known as complexes. Modulations of complexes provide an important component towards understanding function

approaches will facilitate proper information flow, making multi-omics more amendable for emerging and powerful AI tools.

Concluding remarks

Aging and longevity are key application areas for data analytics and biotechnology, with interest from many different parties, including medical doctors, scientists, and engineers. The current euphoria over AI and machine learning needs to be tempered with good sense or else we risk being disappointed, or worse, misled. Model organisms are better suited for big data analytics but might lack relevance because they do not immediately reflect the human condition. To resolve this and to bridge the human–model organism gap will require some finesse. Appropriate use of biological context, critical thinking on multi-omics integration issues, and developing novel validation strategies using synthetic biology approaches are important considerations for advancement.

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