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REVIEW

Towards organism-level systems biology by next-generation genetics and whole-organ cell profiling

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Abstract

The system-level identification and analysis of molecular and cellular networks in mammals can be accelerated by "next-generation" genetics, which is defined as genetics that can achieve desired genetic makeup in a single generation without any animal crossing. We recently established a highly efficient procedure for producing knock-out (KO) mice using the "Triple-CRISPR" method, which targets a single gene by triple gRNAs in the CRISPR/Cas9 system. This procedure achieved an almost perfect KO efficiency (96–100%). We also established a highly efficient procedure, the "ES-mouse" method, for producing knock-in (KI) mice within a single generation. In this method, ES cells were treated with three inhibitors to keep their potency and then injected into 8-cell-stage embryos. These procedures dramatically shortened the time required to produce KO or KI mice from years down to about 3 months. The produced KO and KI mice can also be systematically profiled at a single-cell resolution by the "whole-organ cell profiling," which was realized by tissue-clearing methods, such as CUBIC, and an advanced light-sheet microscopy. The review describes the establishment and application of these technologies above in analyzing the three states (NREM sleep, REM sleep, and awake) of mammalian brains. It also discusses the role of calcium and muscarinic receptors in these states as well as the current challenges and future opportunities in the next-generation mammalian genetics and whole-organ cell profiling for organism-level systems biology.

Keywords Systems biology · Next-generation genetics · Triple CRISPR · ES mouse · Whole-organ cell profiling · Tissue clearing

Introduction

A life system is a system consisting of multiple layers of biological components (e.g., genes, transcripts, and proteins), and the elements between these layers interact with each other (e.g., the central dogma of molecular biology) (Cobb 2017). Living systems are indeed far too complex to be understood based on a linear chain of cause and effect. Systems biology promises to speed up the process of research by bypassing the classical methods and using a holistic approach (Kellenberger 2004). Systems biology is a multidisciplinary field of research, useful for understanding the target biological system. It typically includes four steps: identifying factors (systems identification), analyzing relationships (systems analysis), controlling systems behavior (systems control), and designing and rebuilding systems (systems design) (Kitano 2000, 2002; Ukai and Ueda, 2010). The latter two are categorized as synthetic biology in some cases. About systems biology, many good textbooks and reviews have been published (Ideker et al. 2001; Alon 2019; Davis et al. 2019; Nijhout et al. 2019; Tyson and Novak, 2020). Focusing on oscillators (e.g., cell cycle, somite segmentation clock, and the circadian clock), examples of successful systems biology approaches can be found in the following articles (Ueda et al. 2005; Masamizu et al. 2006; Novak and Tyson, 2008; Ukai-Tadenuma et al. 2008; Tigges et al. 2009; Ferrell et al. 2011; Ukai-Tadenuma et al. 2011; Ode et al. 2017).

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This review explains the possibility of performing organism-level systems biology research on mammals, especially systems identification, which can be attributed to high-throughput quantitative analysis and next-generation genetics. We further discuss the whole-organ cell profiling, which is realized by tissue-clearing methods and light-sheet microscopy. Furthermore, the challenge of achieving systems biology on an organismal level in sleep studies using mice will be presented, and finally, systems biology in humans will be discussed.

Organism-level systems biology

Systems identification

Systems biology focuses on the relationships among players rather than the players themselves. Systems identification is a step towards identifying the players in the target biological phenomenon. The keywords in systems identification are "comprehensiveness" and "quantitative analysis." In order to get a complete picture of the target and understand the relationships among players, it is necessary to identify the players comprehensively. Uniform and high-throughput quantitative analyses support measuring the behavior of the system and analyzing players' weights on the system's behavior (Susaki et al. 2017).

Comprehensiveness

Many important findings in life science have been reported in the past 20 years. The human genome project was a successful pioneer of the big science in life science, and its output, decoded genome sequence, changed researchers' way of thinking, planning, and performing experiments. Nowadays, not only species' genomes (e.g., humans, mice, and rats) (Lander et al. 2001; Venter et al. 2001; Mouse Genome Sequencing et al. 2002; Gibbs et al. 2004; International Human Genome Sequencing 2004; Zoonomia 2020) but also personal genomes are available (Gonzaga-Jauregui et al. 2012; Lupski 2016).

Systems identification is supported by omics approaches. The suffix "-ome" (completion) is used in a variety of biological layers, such as gene (genome), RNA (transcriptome), and protein (proteome) (Joyce and Palsson, 2006). The omics allow researchers to perform non-biased and comprehensive analyses and achieve the real nature of their target biological systems. Previously, researchers were constrained to choose study targets based on existing reports and their inspiration without knowing the whole picture. However, through omics approaches, researchers can choose targets from a known number of candidates. Further, we can test "all" candidates listed (Joyce and Palsson, 2006). Multi-omics is a kind of challenge that elucidates the fundamental features of organisms by integrating various omics data (Hasin et al. 2017). Employing data and models produced from multi-omics methods allows researchers to obtain a comprehensive view of their target molecular pathways, organisms, or diseases, etc. (Nielsen 2017; Pinu et al. 2019). In 2020, Wang et al. discovered a circadian kinome using multi-omics data. The team employed fly heads to perform phosphoproteomics jointly with transcriptomic and proteomic profiling and found 789 phosphorylation sites that displayed circadian oscillations. They also predicted 27 kinases that were potentially involved in the phosphorylation of these sites (Wang et al. 2020).

The understanding of cellular systems was brought by decoding and understanding the perfect set of genes in some respects. The understanding of multicellular units, such as tissues and organisms, is more complicated because tissues and organisms are composed from heterogeneous cell types. Although the understanding of functional gene networks in tissues has been achieved through knockout (KO) mouse studies, there are bottlenecks to raise throughput of the gene modified animal studies. In this sense, we introduce next-generation genetics as mentioned in "Next-generation genetics." To understand components behavior involving the systems, we have to have a "cell catalogue" of the target tissues or organism. In this sense, the methods for figuring out the whole cells in one tissue are needed. One of such key technologies is the tissue clearing as mentioned in "Whole-organ cell profiling."

Next-generation genetics

The bottlenecks

Although investigating mammalian systems biology on an organism-level appears ideal, two bottlenecks exist in the genetic modification and the crossing steps. Traditionally, the process of generating a knock-in (KI) or KO model mouse is arduous and prolonged. Researchers need to first produce their mutations of interest by means of gene targeting, whose efficiency depends on the prolonged rate of spontaneous homologous recombination, and then introduce these mutations to the embryonic stem cells (ESCs) of mice (Capecchi 2005). Another bottleneck occurs in the crossing step. After obtaining the gene-modified ESCs, researchers need to inject these into the blastocysts of the wild-type mice. As a result, chimera mice have both cells derived from the ESCs and wild-type embryos. Typically, two generations are required for these chimera mice (F0) to give rise to the homozygous mutants (F2), which carry two copies of the mutated genes of interest. Such a crossing procedure proceeds slowly and typically demands a period

of over 9 months (3 months/generation × 3 generations) for it to be completed (Fig. 1a).

Furthermore, rapid quantitative phenotyping methods are necessary. In sleep research, we need expertise in surgical techniques and analysis skills to investigate sleep staging. Thus, it is essential to accelerate the process of sleep analysis through quantitative analysis and other innovations.

Quantitative phenotype analysis

To achieve a complete understanding of biological systems at an organism level, establishing quantification analytical methods of the target biological systems' behavior is essential. In chronobiology, the identifications of the *period* gene in fruit flies and the *Clock* gene in mice are great successes brought by the forward genetic mutants screening. These identifications were achieved by counting the infra-red beam interruption (flies) or wheel revolutions (mice) to analyze the circadian rhythm phenotypes of the subjects (Konopka and Benzer, 1971; Reddy et al. 1984; Zehring et al. 1984; Vitaterna et al. 1994; King et al. 1997). Regarding sleep research, Yanagisawa's group performed a forward genetic screening on mice harboring ethyl nitrosourea–derived mutations. Through electroencephalography and electromyography staging, they found two interesting mutants, *Sleepy* (*Sik3*) and *Dreamless* (*Nalcn*) (Funato et al. 2016). International Mouse Phenotype Consortium published their phenotyping results from studying more than 7,500 knockout mice with standardized phenotyping methods. Zhang et al. screened 750 mutant mice lines with the indirect calorimetry datasets and identified five novel genes involved in circadian misalignment (Zhang et al. 2020). Our team developed Snappy Sleep Stager (SSS), a high-performance, non-invasive, and respiration-based fully automated sleep phenotyping system (Sunagawa et al. 2016). Now sleep–wake patterns of 1,152 mice can be tested weekly in our facility.

Triple CRISPR

Over the past decade, efforts have been made to overcome the aforementioned genetic modification and crossing issues. The CRISPR/Cas9 system, which contains a Cas9 DNA endonuclease, a CRISPR RNA (crRNA), and a trans-activating crRNA (tracrRNA), emerged as one of such efforts. The crRNA and tracrRNA were further edited and fused to become a synthetic single-guide RNA (sgRNA) (Deltcheva et al. 2011; Jinek et al. 2012). This technology had effectively raised the efficiency of genetic modification and enabled researchers to cross the bottleneck in genetic modification (Wang et al. 2013; Yang et al. 2013). More advanced than the traditional CRISPR/Cas9 system, the Triple-CRISPR method concurrently utilizes three types of sgRNA for targeting one gene. Our study has revealed that this invention allows the Triple-CRISPR technology to achieve a biallelic KO efficiency of about 100%, which is considerably higher than the efficiency attained by the conventional

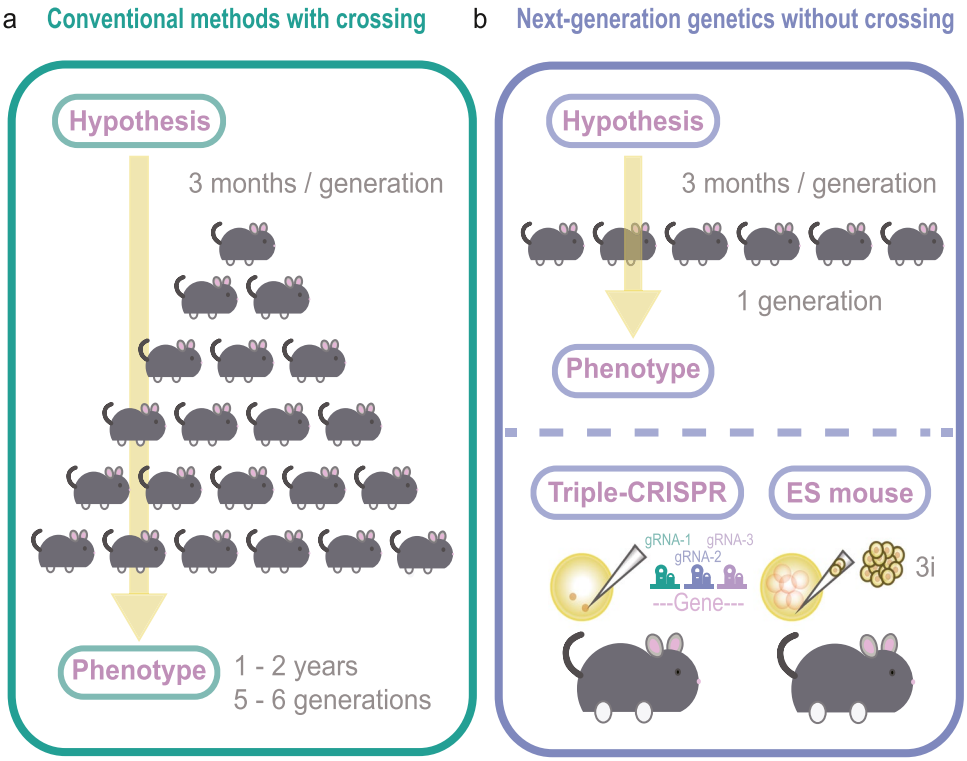

Fig. 1 The next-generation mammalian genetics. **a** Conventional methods with crossing. By conventional methods, more than 1 year is required to obtain enough gene-modified mice and test the hypothesis. This is because crossing is required for preparing mice. **b** The next-generation genetics without crossing. The next-generation mammalian genetics is genetics without crossing. We can test hypotheses after about 3 months because crossing is unnecessary. These are based on the Triple CRISPR and ES mouse methods. Details of the Triple CRISPR (lower left) and ES mouse (lower right) are mentioned in the text

single-targeted CRISPR (Sunagawa et al. 2016). Attributed to such an almost perfect biallelic KO efficiency from the Triple CRISPR, researchers can now address the crossing issue, as they are able to obtain the homozygous mutants at the F0 generation (Fig. 1b). Promisingly, a triple-target CRISPR screen identified *Slc38a4*/*SNAT4* as a critical amino acid transporter for placental development in mice (Matoba et al. 2019). The group also succeeded in generating mice with spermatozoa fully derived from ES mice using this method (Miura et al. 2021).

ES mouse

The ES mouse is almost entirely ESC-derived and carries mutations throughout the body in its F0 generation. ESCs are commonly cultured with leukemia inhibitory factor (LIF) and serum to maintain their potency and preserve them at an undifferentiated stage. There are three inhibitors (3i), the FGF receptor inhibitor, the GSK3 inhibitor, and the ERK inhibitor, which also support the undifferentiated stages of the ESCs (Ying et al. 2008). After being treated with the 3i, these ESCs are then injected into 8-cell stage embryos at high potency, which increases the contribution of ESC-derived cells in the born mice (Kiyonari et al. 2010). Generating diverse kinds of KI ESCs in parallel with the ES mouse technology, Ukai et al. further enhanced the productivity of the KI ESCs. They accomplished a systematic production of various types of the KI ES mice in 2 to 3 months (Ode et al. 2017; Ukai et al. 2017) (Fig. 1b).

Whole-organ cell profiling

Establishing tissue-clearing methods

Tissue opacity results from light scattering and light absorption. The former originates from the heterogeneity of refractive index (RI) in components of biological tissues, such as water, lipids, and proteins. The latter comes from chromophores, such as hemoglobin. There is a hundred-year history of developing chemical reagents to unify RI and decolorize the native pigments (Susaki and Ueda, 2016; Ueda et al. 2020a, 2020b; Tian et al. 2021). The German anatomist Werner Spalteholz was a pioneer of the field. In the 1910s, he developed a method for making human tissue translucent by hydrophobic reagents. Another hydrophobic tissue clearing method, BABB, was reported after decades of silence (Dent et al. 1989). Hydrophilic tissue-clearing methods were reported by Chiang et al. and Tuchin et al. (Chiang et al. 2001; Tuchin et al. 2002). The great success in 3D-fluorescent imaging using clearized tissues by Dodt et al. and Hama et al. (Dodt et al. 2007; Hama et al. 2011) triggered extensive development of several types of tissue clearing methods in the 2010s, such as the hydrophobic 3DISCO and the hydrogel-based CLARITY (Erturk et al. 2012; Chung et al. 2013; Chung et al. 2013). We established hydrophilic CUBIC methods (Susaki et al. 2014; Tainaka et al. 2014). Boyden's group reported Expansion microscopy, which employs a swollen hydrogel to expand tissue isotropically (Chen et al. 2015).

A light-sheet microscope is an appropriate tool to obtain a single-cell resolution image of a whole target organ on a realistic time scale. This microscope uses sheet light illuminations to illuminate the specimen from the side. Researchers can then capture 2D stacks and reconstruct a 3D image in silico (Dodt et al. 2007; Keller and Dodt, 2012; Murakami et al. 2018; Matsumoto et al. 2019; Mano et al. 2021).

Cell profiling at a whole-organ level

Whole-cell profiling in the brain is one of scientist's long-lasting dreams, and tissue clearing methods make this dream come true (Fig. 2). Here, we briefly introduced examples of cell profiling at a whole-organ level. The methods of these cell profiling technologies, including those not mentioned here, are well reviewed elsewhere (Susaki and Ueda 2016; Gradinaru et al. 2018; Mano et al. 2018; Wassie et al. 2019; Parra-Damas and Saura, 2020; Ueda et al. 2020a, 2020b; Molbay et al. 2021; Tian et al. 2021).

Dott et al. first observed mouse brains at a single-cell resolution using organic solvent benzyl alcohol and benzyl benzoate (BABB) with light-sheet microscopy (Dodt et al. 2007). Ertürk et al. also observed the whole mouse brain at a single-cell resolution by developing the 3DISCO (Erturk et al. 2012). Pan et al. clearized tissues from the brain to the spinal cord using uDISCO in mice and rats and imaged neuronal connections and vasculature for the subjects from head to toe for over 7 cm (Pan et al. 2016). Samples treated with hydrophobic reagents shrink in size. The advantage of the hydrophobic method is its rapid and robust clearing, but the disadvantage of this method is its rapid bleaching of endogenously expressed fluorescent proteins. In this regard, uDISCO can preserve fluorescent proteins for several months and make intact organs and rodent bodies transparent (Erturk et al. 2012; Pan et al. 2016). Ke et al. utilized SeeDB to describe the near-complete wiring diagram of sister mitral cells associated with a common glomerulus in the mouse olfactory bulb (Ke et al. 2013). Susaki et al. observed not only mice but also common marmoset's brains using CUBIC methods (Susaki et al. 2014, 2015). Murakami et al. drew a whole-brain atlas at one single-cell resolution using CUBIC-X and generated the CUBIC Atlas, an analytic platform for whole-brain cell profiling (Murakami et al. 2018). This method has been further developed into CUBIC-Cloud (Mano et al. 2021). SeeDB, CUBIC, and CUBIC-X are hydrophilic methods that feature biocompatibility and

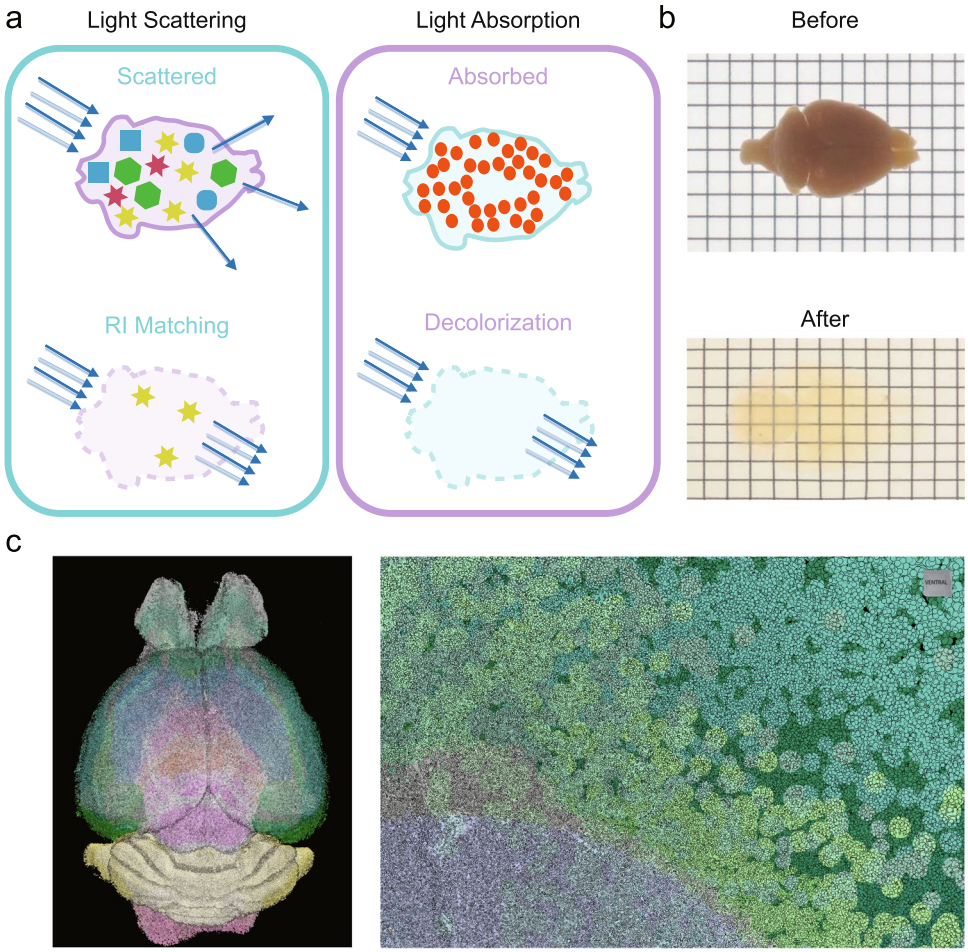

Fig. 2 Whole-organ cell profiling by tissue-clearing. **a** Tissue opacity resulted from light scattering and light absorption. Tissue clearing can be achieved by setting an appropriate refractive index (left) and decolorization of pigments (right). **b** By hydrophilic (e.g., Scale, SeeDB, CUBIC), hydrophobic (e.g., BABB, 3DISCO, uDISO), or hydro-gel-based tissue-clearing methods (e.g., CLARITY, Expansion Microscopy), fixed organs become translucent. Examples of before and after CUBIC-treated brains are shown. Photos are taken by Mr. Shigeta (The University of Tokyo). **c** We can reconstruct organs in silico at a single-cell resolution. As examples, snapshots of a mouse brain reconstructed in silico (CUBIC-Cloud) are shown; the whole brain (left) and an enlarged part of the brain (right). Each color dot represents a single cell. The snapshots are kind gifts from Drs. Matsumoto and Mitani (RIKEN BDR)

biosafety, and these methods require relatively long incubation time for tissue clearization. SeeDB focuses on RI matching, while CUBICs and CUBIC-X use a combination of delipid and RI matching for tissue clearing. In contrast to CUBICs, CUBIC-X has a hydration process, which expands tissues. Inoue et al. succeeded in human brain clearing by rapid delipidation of human brain tissues (Inoue et al. 2019). Recently, Zhao et al. reported tissue clearing of the human brain using SHANEL (Zhao et al. 2020). Hydrophilic SHANEL can be applied to primates, especially humans. The hydrogel-based method, CLARITY, was employed in mouse brain tissue clearing (Chung et al. 2013). SHIELD is a technology from the same family as CLARITY that can be used to accomplish transparency in human tissues, including the brain (Park et al. 2019). CLARITY is an original hydrogel-based tissue clearing method, and SHIELD preserves the fluorescence and antigenicity of proteins as well as the architecture of transcripts and tissues under harsh conditions.

Besides brains, tissue clearing can also be applied to other tissues. In our work, CUBIC and its derivatives were employed to investigate organs, such as the kidney and heart, and the whole body of mice and marmosets (Tainaka et al. 2014; Susaki and Ueda, 2016; Susaki et al. 2020). Belle et al. succeeded in their 3D visualization and analysis of early human development by transparentization of human embryos (gestation weeks (GW) 6 to 8) and human fetuses (GW8.5 to 14). They utilized a combination of whole-mount immunostaining, 3DISCO clearing, and light-sheet imaging during their research (Belle et al. 2017). These suggest that cell profiling at a whole-organ level is now realized not only in animal models but also in humans.

Application in mouse sleep research

Calcium makes mice sleep

By combining SSS with the Triple-CRISPR technology, we were able to perform sleep phenotyping of KO mice covering almost all gene families. A comprehensive KO study through one gene family is now achievable (Sunagawa et al. 2016; Tatsuki et al. 2016; Niwa et al. 2018; Yoshida et al. 2018) (Fig. 3).

Using a simulation approach, Tatsuki et al. identified that Ca^{2+} was a key player for regulating sleep in mice. Our group has developed a computational model based on a

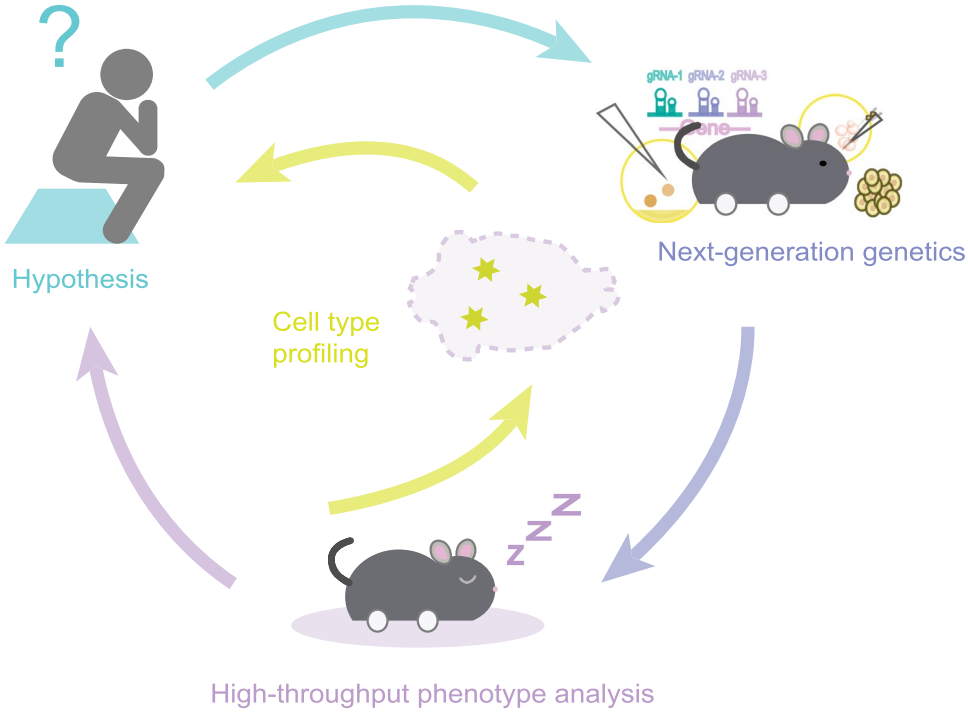

Fig. 3 Applications of next-generation genetics and cell profiling methods. Based on a working hypothesis, researchers genetically perturb mice efficiently using the next-generation genetics. Through high-throughput analysis on their models' phenotypes, researchers are able to test their working hypothesis. Whole-cell profiling may help researchers verify their hypothesis, and spatio-temporal information obtained from cell profiling could improve their hypothesis. In our sleep research, we first hypothesized the critical role of calcium in sleep and then tested our hypothesis by generating a series of KO mice. We systematically analyzed their phenotypes by respiratory-based sleep analyzer afterward

Ca^{2+}-dependent sleep duration hypothesis, and we identified multiple genes that could be potentially involved in the act of sleep. We then decided to screen for key molecules related to sleep generation by testing 21 gene KO mice using the SSS and the Triple-CRISPR methods. Our research discovered that dysfunctional Ca^{2+}-dependent K + channels (*Kcnn2*, *Kcnn3*), voltage-gated Ca^{2+} channels (*Cacna1g*, *Cacna1h*), and Ca^{2+}/calmodulin-dependent kinases (*Camk2a*, *Camk2b*) reduced the duration of sleep in mice. In contrast, dysfunctional plasma membrane Ca^{2+} ATPase (*Atp2b3*) lengthened the sleep time in mice (Tatsuki et al. 2016). In addition, a whole neuron behavioral change by NMDA receptor antagonist (MK-801) was studied using the CUBIC analysis (Tatsuki et al. 2016). We were able to reveal that the impaired NMDA receptors decreased sleep duration while elevating neural excitability in mice. The research above suggests that calcium promotes sleep in mice (Tatsuki et al. 2016). Further studies have indicated the involvement of sleep-promoting kinase phosphorylation, like CaMK2 phosphorylation, which is downstream from the calcium pathway (Ode and Ueda, 2020).

Acetylcholine receptors are needed for REM and NREM sleep

The regulatory role that the cholinergic system plays in mammalian sleep has been widely investigated (Brown et al. 2012). Our research team systematically generated acetylcholine receptors KO mice (11 nicotinic acetylcholine receptors and 5 muscarinic acetylcholine receptors) using the Triple CRISPR and confirmed phenotypes using the SSS method (Niwa et al. 2018). The results of our study suggest that muscarinic acetylcholine receptors M1 and M3 are essential for the regulation of sleep. The KO of *Chrm1* shortened and fragmented REM sleep, while the KO of *Chrm3* reduced NERM sleep durations of the subjects. Strikingly, the *Chrm1* and *Chrm3* double KO mice illustrated a REM-less phenotype. This phenotype was also confirmed by investigating the ES mouse base *Chrm1*/*Chrm3* double KO mice (Niwa et al. 2018). Overall, the next-generation genetics has raised the throughput of in vivo analyses involving genetically modified animals, which has enabled a more efficient system identification in mammalian organism-level systems biology (Fig. 3).

Future directions for organism-level systems biology

From correlation to causality

Humans are the most important and fascinating model animals. However, due to many limitations, systems biological approaches cannot be conducted on humans as we have been able to do on mice (Ukai et al. 2019). Ethically, experimental-motivated genetic modification is not allowed to be conducted on humans (Lanphier et al. 2015; Munsie and Gyngell, 2018). Technically, we cannot perform genetically controlled experiments on humans because of our genetic diversity (Frazer et al. 2009; di Iulio et al. 2018).

Furthermore, life-long observations are difficult to perform as humans have relatively long-life spans (Fig. 4).

Today, we endeavor to establish a complete picture of human systems biology. To accomplish this, it is important to gather multiple layers of research: quantitative behavioral analysis in humans and genome-wide analysis of associated genes (Seshadri et al. 2010; Jansen et al. 2019; Uffelmann et al. 2021). This is in addition to high-throughput model animal phenotyping studies as well as further analysis of key regulators of the target systems at a molecular level in vitro (Fig. 4). Specifically, it is necessary to initially observe and investigate the correlations between human genotypes and phenotypes through large-scale quantitative analyses in humans. Particularly, these analyses have revealed interesting topics worthy of investigation. For example, a genome-wide analysis of insomnia disorder revealed heritability as a significant factor. The research also found a significant locus on Chr 7 (q11.22) among the three ancestral groups studied (European American, African American, and Latino American) and a significant gene-based association for RFX3 on Chr 9 in European American population (Stein et al. 2018). Notably, biologists are able to employ various genome-wide analyses to explore the potential gene components of diseases of their interest such as Alzheimer's disease, HIV disease, stroke, coronary artery disease (CAD), type 2 diabetes, and breast cancer (Stein et al. 2010; Wu et al. 2011; Dichgans et al. 2014; Xue et al. 2018; Ferreira et al. 2019). Additionally, associations found in genome-wide analysis studies could help scientists in the process of selecting genes for further investigation. However, since such correlations observed from human data do not indicate causal relations directly, it is imperative for researchers to conduct rigorous experiments using animal models to further explore the causality between the selected genes and the target diseases of interest (Tam et al. 2019). Moreover, when envisioning the possible genotype–phenotype relationships that contribute to their target systems, biologists from this new era can utilize the various high-throughput next-generation genetic methods to perform phenotyping studies on mice models (Susaki et al. 2017; Ukai et al. 2019). Such research will be completed in vitro and in silico to analyze the identified candidates' molecular characteristics (Ukai et al. 2019).

Identification of the potential regulators of the systems can also guide the process of assessing the target systems.

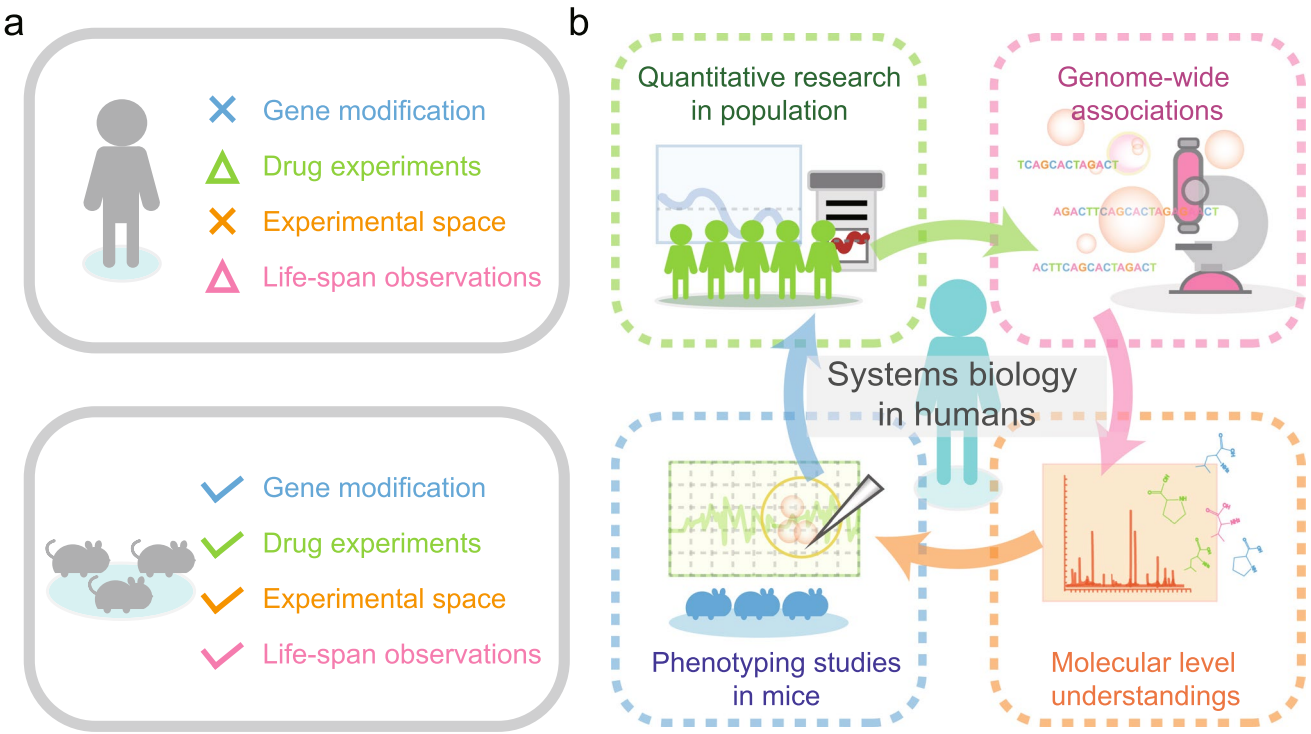

Fig. 4 Systems biology in humans. **a** The difference between humans and mice when being employed as subjects for various biological research and analyses. Regarding the symbols, check marks suggest methods that can be completed on the subjects or advantages when employing the subjects. Triangles stand for methods that can be completed with risks or caution. Crosses indicate methods that cannot be completed on the subjects or disadvantages when employing the subjects. **b** Multiple layers of studies to achieve a system-level understanding of humans. Here, we demonstrate examples of large-scale quantitative research in humans, genome-wide association studies, verification by mouse phenotyping studies, and identification of regulators of the biological systems of interest. Details are shown in the text

Through accumulating these various studies and promoting a comprehensive understanding of this field, biologists will eventually have a broader understanding of their research topic at a systems level (Chuang et al. 2010; Kohl et al. 2010). Such inspiring insights would ultimately contribute to advanced drug discovery and systems-based medicine.

Nature vs. nurture

Systems behavior is defined as both "nature" and "nurture." "Nature" is defined as an individual's genomic information, while "nurture" is defined as the environmental exposure history of the individual (Coll et al. 2003). Such environmental information can accumulate in the body as epigenetic changes or immunological factors, etc. (Tammen et al. 2013).

The methods we have described so far have only revealed the "natural" aspect of the human biological system. Is there any way to reveal the "nurtural" aspect? Epigenome analysis using next-generation sequencers can provide comprehensive information on gene silencing/activation, allowing us to investigate acquired changes in our genome (Ku et al. 2011; Kimura 2013). Also, if we are able to identify the epitopes of all the antibodies present in a single person, we can then learn about the antigen exposure history of the person. This process will also reveal the history of the environments to which the person has been exposed to. Although the development of such a technology is challenging, it is worth future investigation.

Conclusions

Systems biology is an interdisciplinary area of research that aims to comprehensively investigate biological systems. For this review, we employed various examples to illustrate that the "next-generation" genetics could accelerate the process of systems-level identification and analysis. We further explained the challenges that organism-level systems biology research had faced and the general concepts of the "Triple-CRISPR" and ES mouse methods. These two methods have significantly enhanced the efficiency of organism-level systems biology studies. In addition, the roles of calcium and muscarinic receptors in the three states (NREM sleep, REM sleep, and awake) of mammalian brains were investigated using these technologies. We also described ways that the tissue-clearing method, CUBIC, and advanced light-sheet microscopy made cell profiling at a whole-organ level a reality. Although systems biology research in mice is now realizable, such research conducted on humans remains challenging due to ethical and technical issues. Future efforts in combining personal genomics with human systems behavior analysis could shed light on systems biology in humans and systems-based medicine, which would benefit all living beings.

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Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors agree for a publication.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals Not applicable.

Conflict of interest H.R.U. is a co-inventor on a patent covering Triple CRISPR (PCT/JP2015/086259 (pending), patent applicant is RIKEN, other co-inventors are K. Sumiyama, G.A. Sunagawa, M. Ukai-Tadenuma, D. Perrin) and patent applications covering the CUBIC reagents (PCT/JP2014/070618 (pending), patent applicant is RIKEN, other co-inventors are E. A. Susaki and K. Tainaka; PCT/JP2017/016410 (pending), patent applicant is RIKEN, other co-inventors are K. Tainaka and T. Murakami) and a co-founder of CUBICStars Inc.

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