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Division of Program Coordination, Planning, and Strategic Initiatives
Office of Research Infrastructure Programs
Division of Comparative Medicine

Validation of Animal Models and Tools for Biomedical Research
Session X. Report and Discussion of Sessions I–IX

Tuesday, January 19, 2021
Virtual Meeting

Workshop Report

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Executive Summary

The final of 10 sessions of the Virtual Workshop on Validation of Animal Models and Tools for Biomedical Research was held on January 19, 2021. This workshop is intended as a venue to discuss the status and needs regarding the validation of animal models and tools used in biomedical research. Session X drew on the discussions from the previous workshop sessions and focused on the topics of technology and resource obstacles and gaps, as well as new approaches for discussing the values and limitations of animal models for human diseases. The previous session topics addressed invertebrate models; zebrafish models; mouse models; large animal models; non-mouse models; nonhuman primate models; non-zebrafish aquatic models; technologies, phenotyping, and data science for animal models; and vertical integration approaches for preclinical research. In discussion, the participants emphasized the importance of supporting data integration, cross-disciplinary training, and documentation of housing and experimental conditions for reproducibility. They concluded by emphasizing the importance of supporting different model organisms to address different biological questions.

Session Co-Chairs
Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Keith Cheng, M.D., Ph.D., Penn State College of Medicine

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Mary Dickinson, Ph.D., Baylor College of Medicine
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Kent Lloyd, D.V.M., Ph.D., University of California, Davis
Cathleen Lutz, Ph.D., The Jackson Laboratory
Calum MacRae, M.D., Ph.D., Brigham and Women's Hospital
John Morrison, Ph.D., University of California, Davis
David O'Connor, Ph.D., University of Wisconsin–Madison
John Postlethwait, Ph.D., University of Oregon
Crystal Rogers, Ph.D., University of California, Davis
Susan Sanchez, Ph.D., The University of Georgia
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Workshop Report

Opening Remarks
Stephanie Murphy, V.M.D., Ph.D., Director, Division of Comparative Medicine, ORIP
Sige Zou, Ph.D., Coordinator, Program Official, ORIP
Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Keith Cheng, M.D., Ph.D., Penn State College of Medicine

Drs. Stephanie Murphy, Director, Division of Comparative Medicine, Office of Research Infrastructure Programs (ORIP), and Sige Zou, Coordinator, Program Official, ORIP, welcomed the participants. Dr. Zou expressed appreciation to the Organizing Committee, Session Co-Chairs, and ORIP leadership for their efforts in organizing the workshop series. He explained that this meeting is the final in a series of 10 sessions. Dr. Murphy thanked the participants for their engagement and noted that nearly 1,500 attendees have participated in the workshop.

Drs. Murphy and Zou also acknowledged the support of several National Institutes of Health (NIH) Institutes: the National Heart, Lung, and Blood Institute (NHLBI); National Institute on Aging (NIA); National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK); National Institute of General Medical Sciences (NIGMS); and National Institute of Neurological Disorders and Stroke (NINDS). Dr. Murphy reminded the participants that validation of animal models and tools is a critical part of ORIP's trans-NIH efforts. She expressed appreciation for the participants' input.

Dr. Hugo Bellen, Co-Chair, thanked ORIP leadership, the Session Co-Chairs, and speakers for their efforts in supporting the workshop. He stated that the goal of the workshop is to determine comprehensively the research community's needs for validation of animal models and tools within the next 5–10 years. Dr. Bellen noted that the participants have provided input on various topics to help ORIP select areas of support. Additionally, he asked the participants to refrain from discussing grant funding mechanisms.

Summary of Session I on Validation of Invertebrate Models for Preclinical Research
Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Julie Simpson, Ph.D., University of California, Santa Barbara

Drs. Bellen and Julie Simpson presented a summary of the major conclusions from Session I, which focused on the validation of invertebrate models for preclinical research. Dr. Simpson conveyed that studies using invertebrate models (e.g., worms, flies) have provided valuable insight on shared biological pathways in humans. In the session, the speakers and participants highlighted the value of stock centers, which curate knowledge and physical resources for researchers but currently lack sustainable financing. Additionally, gene and protein tagging were emphasized as critical tools that require further technological development. Dr. Simpson stated that knowledge sharing among investigators who work on different model organisms is crucial. Additionally, integrated atlases would enable large-scale analyses of newly generated data; physical storage and enhanced software capabilities, however, are needed. Dr. Bellen underscored that data integration (e.g., genomics, transcriptomics, proteomics, metabolomics) among databases is a crucial area for further development; format consistency remains a challenge for researchers. He highlighted key needs of the *Caenorhabditis elegans* community: non-mutagenic CRISPR, vetting of orthogonal recombinases, multiplex genome engineering strategies, injection robots, and downstream workflows (e.g., automated cell expression identification, automated localization in signature cells, protein co-localization by super-resolution, protein proximity in time and place, binding proteins in time and place), and targeted gene mutagenesis. He noted that these issues also are applicable to fly research. Additionally, Dr. Bellen stated that shared imaging centers would provide a valuable

resource for the research community. He listed the following major needs: stock centers; model organism databases; molecular tagging of all genes in worms and flies using CRISPR; systematic links between genome science and phenomics; technology to produce split proteins based on intein technology; a library of G protein-coupled receptors; nanobody libraries for visualizing proteins and their functional analysis; interrogation of the proteome in its native environment based on proximity labeling to provide tools for mapping the protein interactome and subcellular proteomes in their native environments; combination of single-cell transcriptome profiling, electron microscopy atlases, and light-level imaging of genetically targeted neurons; centralized databases to mine data from other databases; four to six microscopy facilities for isotropic special resolution; whole-brain imaging with molecular contrast and nanoscale resolution; and scanning electron microscopy for large-volume 3D imaging.

Summary of Session II on Validation of Zebrafish Models for Preclinical Research
Rebecca Burdine, Ph.D., Princeton University
William Talbot, Ph.D., Stanford University

Drs. Rebecca Burdine and William Talbot presented a summary of the major conclusions from Session II, which focused on the validation of zebrafish models for preclinical research. In this session, the speakers highlighted the wealth of previously developed zebrafish models for human disease. Dr. Talbot emphasized that zebrafish mutations can be used for validated models for a wide array of diseases, gene discovery, and elucidation of gene function. Additionally, chemical screens with zebrafish mutants can identify new molecules with therapeutic potential. Dr. Talbot highlighted major recommendations: provide strong, continued support for the Zebrafish Information Network (ZFIN) and the Zebrafish International Resource Center (ZIRC); support the development and application of technologies to modify the genome, humanize the zebrafish genome, and knock versatile tags into zebrafish genes; support centers to exploit fully the potential of chemical screens in zebrafish to pursue new therapeutics (also prioritize centers to facilitate imaging and genome editing); provide support for new screens and new phenotyping technology in zebrafish; develop programs to support collaborations between clinicians and scientists to facilitate preclinical studies in disease models; and support the development of nanobodies that recognize zebrafish proteins of interest.

Summary of Session III on Validation of Mouse Models for Preclinical Research
Cathleen Lutz, Ph.D., The Jackson Laboratory
Douglas Wallace, Ph.D., Children's Hospital of Philadelphia

Drs. Cathleen Lutz and Douglas Wallace presented a summary of the major conclusions from Session III, which focused on the validation of mouse models for preclinical research. Dr. Wallace provided historical context on the use of mouse models, which are well established within the research community. He outlined the benefits of mouse models, which include similarity to the biology and genetics of humans; small size, low cost, and convenient housing and maintenance; a genome that is easy to manipulate; resources and reagents available to ensure reproducibility in laboratories around the world; and a strong track record of models for face, construct, and predictive validity for many monogenic diseases. In the session, Dr. Craig Franklin spoke on the connection between gut microbiota and validation of rodent models; several factors (e.g., diet, environment, mitochondrial DNA) are likely to influence phenotypes. Dr. Leonard Shultz spoke on his work on the development of humanized mice models for diseases, which can be applied across numerous research areas (e.g., hematology, immunology, cancer, regenerative medicine, diabetes, SARS-CoV-2 infection). Dr. Catherine Kaczorowski discussed the application of humanized mouse models in personalized medicine to treat Alzheimer's disease. Dr. Kenneth Chien presented on the use of humanized mouse models for next-generation cardiovascular regenerative therapeutics, which his group is applying to the enrichment of human ventricular progenitor cells. Dr. Wallace listed challenges for mouse validation: rigor and reproducibility, conservation of physiology

between mice and humans, modeling complex diseases, accounting for environmental and microbiotic factors, models for diseases of aging, and introduction of genetic diversity. Suggestions for improving mouse reproducibility included consistency of source and reporting, confirmation of genetic and metabolic system conservation, use of humanized mice, consideration of the environment, storage of fecal pellets for microbiome analysis, and identification of relevant limiting factors to lifespan. Dr. Lutz underscored the value of mouse models as a tool to understand gene–environment interactions and population genetics. She conveyed the importance of maximizing translatability to human disease.

Summary of Session IV on Validation of Large Animal Models for Preclinical Research
Susan Sanchez, Ph.D., The University of Georgia
Jill Weimer, Ph.D., Sanford Research

Drs. Susan Sanchez and Jill Weimer presented a summary of the major conclusions from Session IV, which focused on the validation of large animal models for preclinical research. Dr. Weimer noted that access to husbandry and trained staff are crucial needs that align with discussions across all workshop sessions. Dr. Sanchez outlined challenges for validation of large animal models: developing a "Rosetta Stone" of animal models (i.e., standardization of language, definitions, and required validation data), supporting tissue banks (i.e., tissue and sample characterization), using magnetic resonance imaging and computed tomography (i.e., for vertical integration), promoting standardized methodology and reporting (i.e., for vertical integration), developing molecular reagents for species (i.e., for vertical integration), identifying naturally occurring models (i.e., for vertical integration and validation), and promoting training for veterinarians and future researchers in the complexities of using large animal models for research. Current needs include extensive housing facilities, research networks, imaging core facilities, genetic core facilities and tissue repositories (e.g., genomics, phenomics), large animal core facilities, and research support around client-owned animals (e.g., the Clinical and Translational Science Awards One Health Alliance).

Summary of Session V on Validation of Non-Mouse Models for Preclinical Research
Mary Dickinson, Ph.D., Baylor College of Medicine
Kent Lloyd, D.V.M., Ph.D., University of California, Davis

Drs. Mary Dickinson and Kent Lloyd presented a summary of the major conclusions from Session V, which focused on the validation of non-mouse models for preclinical research. Dr. Dickinson explained that non-mouse models bridge a gap between highly proliferative mouse models and large animal models and can provide new insights on human physiology. She noted that basic development of these models is needed. Advantages of non-mouse models include that they can exhibit traits not found in mouse models, they sometimes exhibit closer physiology to humans than other models, they sometimes reproduce specific disorders more accurately, and they are small enough for feasible husbandry. Limitations of non-mouse models include high cost, lack of inbred strains or complete genome information, need for specialized skillsets and adapted methods, and lack of common reagents and resource sharing across laboratories. Dr. Dickinson outlined specific examples of advantageous models. First, rabbits are ideal models for muscular and cardiovascular disorders because of their large size, short gestation period, high proliferation, and ease of genetic manipulation. Additionally, naked mole rats exhibit hypoxia and hypercapnia intolerance, imperviousness to painful stimuli, unusual immune cell populations, resistance to such conditions as cancer, cardiovascular diseases, neurodegeneration, and abrogated aging. Further, ferrets experience diseases affecting the lung, liver, and pancreas (e.g., cystic fibrosis, pancreatitis) and viral infections (e.g., flu, coronaviruses, respiratory syncytial virus). Additionally, rats exhibit a neural physiology close to humans. Lastly, hamsters experience infectious diseases similar to humans (e.g., COVID-19). Current needs include the following: resources to support the generation and distribution of non-mouse models, molecular tools and reagents (e.g., specific antibodies) for model

validation, support for genome sequencing and annotation, public support for investigators, support for model standardization, and facilitation of collaborative efforts and data sharing.

Summary of Session VI on Validation of Nonhuman Primate Models for Preclinical Research
John Morrison, Ph.D., University of California, Davis
David O'Connor, Ph.D., University of Wisconsin–Madison

Drs. John Morrison and David O'Connor presented a summary of the major conclusions from Session VI, which focused on the validation of nonhuman primate (NHP) models for preclinical research. Seven National Primate Research Centers (NPRCs) comprise a national consortium and receive funding from ORIP. Dr. Morrison explained that the NPRCs provide unique living conditions; outdoor corrals provide a natural setting for the breeding colony. The following NPRC-focused recommendations were identified: (1) build resources to effectively anticipate and respond to the next potential infectious disease pandemic; (2) expand breeding colonies while improving documentation of pedigrees; (3) characterize the genetics of all monkeys using multiple technologies (e.g., genomics, transcriptomics, proteomics, metabolomics, microbiome analysis) throughout the lifespan; (4) further develop specialized colonies (e.g., aged monkeys) and expand their availability to collaborative teams; (5) enhance capacity for evaluation of phenotypes based on human disease phenotype and encourage phenotype experts not currently using primates to assist; and (6) enhance mechanisms for tissue sharing, digitization of primate pathology, and remote validation within the NHP community and for cross-validation with human and other model systems. Dr. Morrison outlined additional recommendations for ORIP: (1) build capacity for breeding and sharing live animals and support large, collaborative teams for gene-edited models; (2) involve the NPRCs in the breeding, maintenance, and veterinary care of gene-edited NHP models with complex phenotypes developed in university laboratories; (3) identify and breed NHPs with spontaneous disease-causing mutations for gene discovery and modeling and expand information sharing; (4) facilitate the development and implementation of *in vivo* imaging and other biomarkers in NHPs that mirror human biomarkers and reflect disease progression; (5) develop in-cage voluntary testing facilities that do not require transport or restraint; (6) inform the public about the importance of NHP models; and (7) enhance specialized training opportunities for veterinarians and scientists committed to NHP research.
Dr. O'Connor noted that the Pfizer-BioNTech, Moderna, and AstraZeneca COVID-19 vaccines were enabled through NHP research. He also emphasized that both surge and supply investments are needed to allow NHP researchers to respond quickly to new challenges.

Summary of Session VII on Validation of Non-Zebrafish Aquatic Models for Preclinical Research
John Postlethwait, Ph.D., University of Oregon
Crystal Rogers, Ph.D., University of California, Davis

Drs. John Postlethwait and Crystal Rogers presented a summary of the major conclusions from Session VII, which focused on the validation of non-zebrafish aquatic models for preclinical research. Topics of discussion included cavefish, platyfish melanoma, the frog neural crest and disease, the stickleback microbiome, regeneration in axolotls, deep phylogenetic mapping in closely related fishes, and sea slug neuroscience and behavior. Dr. Postlethwait presented an overview on the use of evolutionary mutant models for human disease, which use nature to select mutant genotypes that are likely to be useful in a particular context. Cavefish, for example, display traits that reflect their adaptation (e.g., high blood sugar, glucose intolerance, insulin resistance) but exhibit metabolic resistance. Compensations in evolutionary models often can inform therapeutic approaches in humans. The validation of evolutionary models involves face validity (i.e., replication of human disease clinical findings), construct validity (i.e., reflection of mechanisms of human disease), and predictive validity (i.e., prediction of unknown aspects of human disease). Several gaps were identified for face validity (e.g., identification of corresponding cell types, visualization of phenotypes at high resolution, standardized assays across the animals), construct validity (e.g., identification of orthologous genes,

identification of orthologous non-coding elements, identification of epigenetic marks, unification of gene nomenclature, comparison of gene expression patterns, location of intracellular proteins, reaction of pharmacological agents), and predictive validity (e.g., disruption of gene activity specificity, replacement of native genes with human alleles). Essential components of vertical integration include orthologous genes, orthologous non-coding elements, orthologous epigenetic marks, gene nomenclature unity, corresponding cell types, comparative gene expression, intracellular protein location, and comparative phenotypes. Additionally, horizontal integration is needed. Workshop participants stated that needs for data integration include centralized databases (i.e., similar to ZFIN) for other aquatic models. Valuable technologies include precise replacement of native sequences with human sequences, protein localization tools (e.g., validated antibodies), and spatial gene expression at the single-cell level. Additionally, workshop participants noted that researchers often are focused solely on their specific question of interest and fail to consider pleiotropic factors in disease; platforms (i.e., similar to the International Mouse Phenotyping Consortium) are needed to facilitate comprehensive whole-organism studies. Stock centers play a crucial role in providing genetic resources and phenomic tools for horizontal integration; participants proposed expanding stock centers to include data from multiple species (e.g., killifish, medaka). They also emphasized that the biennial Aquatic Models of Human Disease (AQMHD) conference facilitates training workshops, community-building opportunities, and dissemination of new advances. The participants also emphasized the value of informatics (e.g., annotated genomes), vertical integration platforms (e.g., genomics, transcriptomics, proteomics, metabolomics, phenomics, anatomical), stock centers, research conferences, and molecular tools (e.g., human gene knock-ins, verified antibodies, vectors, spatial transcytosis) for researchers.

Summary of Session VIII on Technologies, Phenotyping, and Data Science for Animal Models
Keith Cheng, M.D., Ph.D., Penn State College of Medicine
Stephen Ekker, Ph.D., Mayo Clinic

Drs. Keith Cheng, Co-Chair, and Stephen Ekker presented a summary of the major conclusions from Session VIII, which focused on technologies, phenotyping, and data science for animal models. Dr. Cheng provided an overview of the session agenda and explained that many of the discussions were concordant with those in other sessions. Topics of discussion included multiplex and intravital imaging, real-time genotyping and phenotyping in live embryos, clinician and patient roles, machine learning for data integration, and genome illumination using protein trap mutagenesis. He highlighted previously noted technologies, including imaging centers, single-cell transcriptomics, emerging models for human disease, genome editing and targeted gene tagging, nanobodies, whole-organism studies (e.g., forward, reverse, and chemical screens), evolutionary models, phenotyping tools, collaborative tools, and data integration. Genome editing's benefits are maximized by cross-usage of vectors and integration of results across organisms and disciplines. Dr. Cheng emphasized the importance of considering and unifying the consideration of phenotypes in the clinic and laboratory models of biology and disease. Principles of validation and research enrichment will require the development of tools for quantitative tissue phenotyping; tools for sharing of full 2D and 3D images that allow independent validation and discovery (i.e., in contrast to the current tendency for "postage stamp" views of single fields); a "Rosetta Stone" atlas for comparative tissue studies; 3D tissue structures; and data integration at multiple scales inclusive of emerging and evolving -omics technologies, enhanced by machine learning and artificial intelligence (AI). Multiple groups agreed that inclusion of human data for comparison will be critical. Several recommendations were posed: integration via computational abstractions of biological entities across length scales and modalities, cross-disciplinary collaboration mediated by those computational abstractions, ontologies and machine learning for establishing associations across species and across scales, computational workflows to document and enable broader use of open-source tools for all steps of analyses, and multiple biological and computational validations and cross-validations using different data sources. Additionally, scientific data management practices were discussed that enable enhanced fidelity of expensive data acquisition, transparency and reproducibility, and broad empowerment of non-experts

to perform and add data analysis. Current limitations for these practices include funding, time, teaching and training, and lack of agreed-on standards. Dr. Cheng concluded by emphasizing that cell, organismal, and tissue phenotypes cannot be predicted using molecular tools without spatial context within the whole organism. He outlined visions for the field within the next 5 years (e.g., a reference atlas across model systems, a cell-based anchor for multiscale multi-omics) and 10 years (e.g., linking molecular pathways, integrated multiscale multi-omics in cell tissue and organismal contexts, toxicological genomics, tissue diagnostics, AI). Mechanisms for documenting and sharing of spatial context for molecular and tissue phenotype were emphasized as critical enhancements that would greatly benefit validation, integration, and discovery in animal models for understanding human biology and disease.

Summary of Session IX on Vertical Integration Approach for Preclinical Research
Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Calum MacRae, M.D., Ph.D., Brigham and Women's Hospital

Drs. Bellen and Calum MacRae presented a summary of the major conclusions from Session IX, which focused on vertical integration approaches for preclinical research. Drs. Bellen and Zhandong Liu presented on the use of model organisms for rare disease diagnosis and therapeutic development through vertical integration. They highlighted the Model organism Aggregated Resources for Rare Variant ExpLoration (MARRVEL), which was established to integrate genetic and genomic information from humans and model organisms to aid in human variant prioritization and experimental design for rare diseases research. Dr. John B. Hogenesch discussed his group's work on data integration for studies of sleep and rhythm at the cellular and organismal levels. Dr. Olga Troyanskaya presented on cross-organism research for human disease studies, highlighting the importance of mapping genomes and phenomes for understanding human disease. Dr. Peter Robinson highlighted the role of the Monarch Initiative in supporting ontology-driven representation of biomedical data (i.e., knowledge graphs). Dr. Rada Savic presented on cross-species integration approaches in drug development for infectious diseases, highlighting tuberculosis as a case example. Informatic strategies to fully support vertical integration include dynamic modeling, network analysis, machine learning (ML) and AI, and "black-box" strategies. For each of these strategies, computational biologists identified the need for larger volumes of standardized phenotypes at cellular and organismal levels. The need for informatics tools (e.g., prediction of the best organism for specific biological questions, platforms to link multidisciplinary teams) was emphasized. Two broad themes—decoding genomes (e.g., at the level of individual alleles, for both gain and loss of function) and decoding biological circuits (e.g., iterative computational modeling with empiric experimentation)—emerged during the session. Necessary experimental tools include deep mutational scanning programs (e.g., cellular and functional assays, saturation mutagenesis, sequencing) and phenotypic assays (e.g., cells through phyla to humans). Several participants noted that semantics is useful but insufficient for phenotypic integration. Additionally, the need for robust cellular or tissue-level phenotypes was emphasized. Overarching themes from the discussion included shared structured data collection (e.g., minimal data sets; shared genotypes, phenotypes, and perturbations; phenotyping by design), program innovation (e.g., collaborative programs, innovative clinical programs, reimagining relationships, new platforms for the completion of comprehensive data sets), bidirectional translation (e.g., access to fundamental pathway expertise, long-term support for transition of clinical observations to basic science), and funding-associated innovations (e.g., support for databases and stock centers, testing approaches, testing in different disease areas, cross-disciplinary education). General concerns included identifying missing data, developing approaches to systematically filling data gaps, leveraging fully exome or genome data in all experiments, aligning mandates across different types of NIH research, ensuring vertical integration is part of programmatic design, defining best approaches, and identifying strategies that generate insights that can be generalized across species.

Group Discussion
Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Keith Cheng, M.D., Ph.D., Penn State College of Medicine

Dr. Bellen asked the participants to identify important topics and concepts that were not included in the previous discussions. Dr. Wallace emphasized the importance of considering physiological factors for vertical integration. Dr. Bellen agreed but noted that this approach represents horizontal integration and thus involves a different approach.

Dr. Erik Jorgensen commented on the importance of defining the molecular topology of the cell using electron microscopy to explore organelles and associated proteins. Dr. Cheng noted that these efforts are being pursued in the pancreas. Dr. Bellen suggested identifying principles and tools needed for development in this area. He also recommended focusing on select tissues before expanding to whole-organism studies. Dr. Cheng added that statistical variation in phenotype must be considered.

Dr. Morrison highlighted the importance of housing and environmental conditions; for example, single-housed and group-housed NHPs are likely to display different disease phenotypes. He acknowledged the complexity of this issue, particularly for other organisms (e.g., zebrafish). Dr. MacRae added that recent data have demonstrated that caging conditions affect hypertension in rat models.

Dr. Jessica Whited asked whether the NIH could require that investigators report experimental and housing conditions in their publications. She emphasized that recent studies have demonstrated the effect of pathogen exposure on phenotype in mice. Dr. Sanchez stated that journals could require these data for publication. Dr. Jeffrey Rogers suggested imposing a requirement that this information be submitted to databases as metadata.

Dr. Burdine underscored the importance of databases for integration; support for these resources is crucial. Dr. Peter Robinson agreed that improved, comprehensive databases are needed. Dr. Jessica Bolker added that ORIP can support skillset development and cross-training for database integration; a gap exists between biologists and informaticians. Dr. Bellen noted that several databases support cross-training. Financial support often is uncertain, however, and retention is challenging. Drs. Bellen and Cheng proposed that the NIH promote workshops and educational outreach efforts.

Dr. Bellen noted that small laboratories play an important role in vertical and horizontal integration; collaborative efforts among these groups are needed. Dr. Burdine expressed support for collaborations that do not necessitate prior relationships between investigators and clinicians. Dr. Bellen explained that MARRVEL (marrvel.org) facilitates efforts in this area.

Dr. Burdine expressed the need for investigators to support one another's model organisms; this issue is particularly critical for support of early-career investigators. Dr. Bellen agreed, noting that a diverse array of animal models is needed to address different biological questions. He underscored the importance of supporting collaborations across different model organisms. Dr. Cheng concluded by challenging the participants to write constructive peer reviews.

Additional Comments

In the Zoom chat, Dr. Bolker suggested promoting more opportunities for early-career researchers to learn about the range of models available and how they might select the most appropriate model(s) to address their questions of interest. She noted that this topic could be addressed at scientific conferences (e.g., AQMHD). Dr. Bolker also proposed that ORIP support a postdoctoral fellowship program on

shared infrastructure to support researchers who want to work with databases (e.g., structure, management, integration).

Dr. Crystal Rogers noted that often, only select models are supported by grant application reviewers and the NIH. A broad array of models is needed for vertical and horizontal integration. She also emphasized that established leaders in the field have an obligation to support new and transformational ideas.

Dr. O'Connor underscored the value of Zoom meetings, which enhance accessibility and diversity among participants while minimizing overall environmental impact and cost. He suggested that ORIP and the NIH promote an initiative to prioritize virtual meetings.

Summary and Suggestions

The participants of Session X drew on the discussions from the previous workshop sessions and focused on the topics of technology and resource obstacles and gaps, as well as new approaches for discussing the values and limitations of animal models for human diseases.

The purpose of the workshop was to assess the needs for tools and resources in validation of animal models for human disease research. The expectations for validation of animal models vary widely depending on the species with respect to human biology. Many invertebrate organisms (e.g., worms, flies) are relevant to genes, pathways, and mechanisms discovery. For other species that are closely related to humans (e.g., primates, rodents), experiments are costly and expectations (i.e., with respect to validation) are increased. The discussants also stressed the importance of supporting a diverse set of animal models to tackle different questions that are relevant to human biology.

The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- Sophisticated imaging facilities (i.e., broad accessibility inclusive of tools for visualization and integrative analytics)
- Improvement of molecular and genetic technologies, including gene-editing technology (i.e., for large-scale mutating and tagging genes, developing humanized animal models, generating nanoantibodies to multiple proteins in model organisms, and detecting interacting proteins in cells and organelles)
- Systematically phenotyping animal models at multiple levels (e.g., single-cell transcriptomics, proteomics, tissue, organ, cell morphology that is inclusive of organismal context, metabolomics, behaviors)
- Screening technologies (i.e., for facilitating high-throughput genetic and chemical screens)
- Stock centers
- Model organism databases (i.e., for data integration and comparisons between animal models and humans)
- AI strategies that allow user-friendly informatic searches and integrative mining of bioinformatic and phenomic data in various databases (e.g., model organism databases, phenotype databases)
- Standardization and reporting of genetic background of strains, housing condition, and environmental conditions (i.e., to promote reproducibility)

- Collaboration among teams with expertise in different model organisms and human biology (i.e., vertical integration) and different disciplines (i.e., horizontal integration) as components of "deep integration"
- Training opportunities for the next generation of scientists (i.e., with respect to vertical and horizontal integration)

Appendix A: Meeting Agenda

Session X. Report and Discussion of Sessions I–IX
1:00–4:00 p.m. EST
January 19, 2021

Chairs
Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Keith Cheng, M.D., Ph.D., Penn State College of Medicine

1:00–1:10 p.m. **Opening Remarks**
Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Keith Cheng, M.D., Ph.D., Penn State College of Medicine
Stephanie Murphy, V.M.D., Ph.D., Director, Division of Comparative Medicine, Office of Research Infrastructure Programs (ORIP)
Sige Zou, Ph.D., Coordinator, Program Official, ORIP

1:10–2:40 p.m. **Presentations**

Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Julie Simpson, Ph.D., University of California, Santa Barbara
Summary of Session I on Validation of Invertebrate Models for Preclinical Research

Rebecca Burdine, Ph.D., Princeton University
William Talbot, Ph.D., Stanford University
Summary of Session II on Validation of Zebrafish Models for Preclinical Research

Cathleen Lutz, Ph.D., The Jackson Laboratory
Douglas Wallace, Ph.D., Children's Hospital of Philadelphia
Summary of Session III on Validation of Mouse Models for Preclinical Research

Susan Sanchez, Ph.D., The University of Georgia
Jill Weimer, Ph.D., Sanford Research
Summary of Session IV on Validation of Large Animal Models for Preclinical Research

Mary Dickinson, Ph.D., Baylor College of Medicine
Kent Lloyd, D.V.M., Ph.D., University of California, Davis
Summary of Session V on Validation of Non-Mouse Models for Preclinical Research

John Morrison, Ph.D., University of California, Davis
David O'Connor, Ph.D., University of Wisconsin–Madison
Summary of Session VI on Validation of Nonhuman Primate Models for Preclinical Research

John Postlethwait, Ph.D., University of Oregon
Crystal Rogers, Ph.D., University of California, Davis
Summary of Session VII on Validation of Non-Zebrafish Aquatic Models for Preclinical Research

Keith Cheng, M.D., Ph.D., Penn State College of Medicine
Stephen Ekker, Ph.D., Mayo Clinic
Summary of Session VIII on Technologies, Phenotyping, and Data Science for Animal Models

Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Calum MacRae, M.D., Ph.D., Brigham and Women's Hospital
Summary of Session IX on Vertical Integration Approach for Preclinical Research

2:40–4:00 p.m. **Group Discussion**

Appendix B: Discussants List

Session X. Report and Discussion of Sessions I–IX
1:00–4:00 p.m. EST
January 19, 2021

Alan Attie, Ph.D., University of Wisconsin–Madison
Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Jessica Bolker, Ph.D., University of New Hampshire
Rebecca Burdine, Ph.D., Princeton University
Michael Chang, Ph.D., Office of Research Infrastructure Programs (ORIP)
Marc Charette, Ph.D., National Heart, Lung, and Blood Institute (NHLBI)
Daofen Chen, Ph.D., National Institute of Neurological Disorders and Stroke (NINDS)
Keith Cheng, M.D., Ph.D., Penn State College of Medicine
Miguel Contreras, Ph.D., ORIP
Kevin Cook, Ph.D., Indiana University
Mary Dickinson, Ph.D., Baylor College of Medicine
Gerald Downes, Ph.D., University of Massachusetts Amherst
Stephen Ekker, Ph.D., Mayo Clinic
Lynne Fieber, Ph.D., University of Miami Rosenstiel School
Scott Fraser, Ph.D., University of Southern California
Bruce Fuchs, Ph.D., ORIP
Franziska B. Grieder, D.V.M., Ph.D., ORIP
Amelie Gubitz, Ph.D., NINDS
Matthew Harris, Ph.D., Harvard Medical School
Marko Horb, Ph.D., Marine Biological Laboratory
Paul Johnson, Ph.D., Emory University
Erik Jorgensen, Ph.D., The University of Utah
Thomas Kaufman, Ph.D., Indiana University
Lisa Schwartz Longacre, Ph.D., NHLBI
Cathleen Lutz, Ph.D., The Jackson Laboratory
Calum MacRae, M.D., Ph.D., Brigham and Women's Hospital
Kathryn Milligan-Myhre, Ph.D., University of Connecticut
D.P. Mohapatra, Ph.D., NINDS
Manuel Moro, D.V.M., National Institute on Aging
John Morrison, Ph.D., University of California, Davis
Stephanie Murphy, V.M.D., Ph.D., ORIP
Peter Nghiem, D.V.M., Ph.D., Texas A&M University
David O'Connor, Ph.D., University of Wisconsin–Madison
Norbert Perrimon, Ph.D., Harvard Medical School
John Postlethwait, Ph.D., University of Oregon
Randall Prather, Ph.D., University of Missouri
Peter Robinson, M.D., The Jackson Laboratory
Crystal Rogers, Ph.D., University of California, Davis
Jeffrey Rogers, Ph.D., Baylor College of Medicine
Rebecca Roof, Ph.D., NINDS
Susan Sanchez, Ph.D., The University of Georgia
Manfred Schartl, Ph.D., *Xiphophorus* Genetic Stock Center, Texas State University
Michael Schmale, Ph.D., University of Miami
Leonard Shultz, Ph.D., The Jackson Laboratory

Julie Simpson, Ph.D., University of California, Santa Barbara
William Talbot, Ph.D., Stanford University
Elly Tanaka, Ph.D., Research Institute of Molecular Pathology
Zoltan Varga, Ph.D., University of Oregon
Stephen Voss, Ph.D., University of Kentucky
Douglas Wallace, Ph.D., Children's Hospital of Philadelphia
Meng Wang, Ph.D., Baylor College of Medicine
Jill Weimer, Ph.D., Sanford Research
Benjamin White, Ph.D., National Institute of Mental Health
Jessica Whited, Ph.D., Harvard University
Bo Zhang, Ph.D., Peking University
Xiaoli Zhao, Ph.D., National Institute of General Medical Sciences
Sige Zou, Ph.D., ORIP

Appendix C: Chairs' Written Session Summaries

Following the workshop, the Co-Chairs of Sessions I–IX prepared summaries of their sessions highlighting major conclusions and suggestions. The workshop consisted of the following sessions:

- Session I. Validation of Invertebrate Models for Preclinical Research
- Session II. Validation of Zebrafish Models for Preclinical Research
- Session III. Validation of Mouse Models for Preclinical Research
- Session IV. Validation of Large Animal Models for Preclinical Research
- Session V. Validation of Non-Mouse Models for Preclinical Research
- Session VI. Validation of Nonhuman Primate Models for Preclinical Research
- Session VII. Validation of Non-Zebrafish Aquatic Models for Preclinical Research
- Session VIII. Technologies, Phenotyping, and Data Science for Animal Models
- Session IX. Vertical Integration Approaches for Preclinical Research

Chair Summary of Session I on Validation of Invertebrate Models for Preclinical Research

Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine
Julie Simpson, Ph.D., University of California, Santa Barbara

The participants of Session I discussed and proposed resources and technologies for the validation of invertebrate models in biomedical research for ORIP and the NIH.

A. FlyBase and WormBase

The greatest priorities in the invertebrate model organism (MO) communities are, without question, stock centers and species-specific databases. These databases are queried millions of times per year and are a lifeline to researchers. They are essential for sharing information among research groups and are critical for establishing connections among research communities. Their support is in jeopardy.

B. The *Caenorhabditis* Genetics Center and the Bloomington *Drosophila* Stock Center

Stock centers are critical to MO-based biomedical research. As the requirements of biomedical research have changed, so have the roles of stock centers; new needs include genome-wide collections of stocks, greater stock complexity, user education on genetic techniques, and stock uses. Centralized maintenance of refererence stock collections improves fidelity and is more cost-effective. Alternatively, stocks could be housed in individual laboratories; this approach, however, leads to duplication of effort, shipping delays, and significant stock loss. As more stocks are generated and private projects are completed, the need for stock centers will increase. Larger collection sizes, more intense information, and website management demands, and added educational efforts should be met with increased NIH support—both from ORIP and from other NIH Institutes, Centers, and Offices. A strong infusion of grant funding to strengthen stock center resources is suggested.

C. *Caenorhabditis elegans* Methods and Resources

Understanding the function of proteins and pathways in cells requires knowledge of the protein's precise (1) localization in the cell, (2) binding interactions, and (3) temporal control. Functional outputs can be determined using methods to acutely activate or disable protein function.

- ***Localization*.** New methods are needed to localize proteins on a scale meaningful to a protein—that is, using methods to wed protein tags (e.g., fluorescent proteins [FPs]) to electron microscopy.
- ***Binding interactions*.** Proteins change partners when pathways are activated. Improved methods and resources are needed to identify complete lists of binding partners that are cell-specific (e.g., better pulldowns, mass spectrometry tools, proximity labels).
- ***Temporal control*.** Location and interactions of a protein must be reported on temporal scales that encompass both broad timescales (e.g., hours, days) for development processes and shorter timescales (e.g., seconds, milliseconds) during signaling.
- ***Perturbation*.** Genetic and pharmacological perturbations act on slow timescales and are crippled by phenotypes that are far downstream of the protein of interest or pleiotropies caused by cell non-autonomous effects. Genetic tags that can acutely inactivate a protein (e.g., degrons) must be developed. Alternatively, tags can be designed that release a protein from a stalled state via light activation or protein release (e.g., retention using selective hooks).
- ***Genome engineering*.** All these tools require protein tagging. An ambitious goal is to tag every gene in the genome with a functional tag (e.g., FPs, dye for super-resolution microscopy, tags for affinity purification, degrons for perturbation studies). These tags will require error-free and

efficient methods for genome engineering (e.g., improved CRISPR). CRISPR-based strategies also can create libraries of worms in which every gene can be manipulated further. This effort might be possible using high-throughput multiplex CRISPR by leveraging the generation of multicopy arrays that contain guide RNAs and tagging templates.

A modular approach would be optimal. For example, each gene could be modified with a cassette exchange target site. Next, the gene could be modified using a specific tag and changed readily when new tags are invented. Such a system would require error-free recombinases for the cassette exchange. Additional recombinases (i.e., other than Flp and Cre) are needed in worms. Generation of transgenics represents a rate-limiting step in worm research. High-throughput methods (e.g., injection robots that work on a broad range of MOs) must be developed.

The tools described are important for all of aspects of biology. In flies and worms, however, the unspoken multiplier for such tools is that these organisms are amenable to genetic screens. The pathways that biological and biomedical researchers study have been elucidated largely in genetic model organisms. Tools that enable the activation or inactivation of pathways in a cell-specific manner will create new, unlimited opportunities for genetic screens. These efforts will, in turn, reveal previously unexplored pathways, and their roles in human health and disease then can be characterized.

Although some aspects of molecular engineering are broad—such as deciding where to split a protein into functional halves (e.g., GAL4, green fluorescent protein [GFP], inteins)—others require species-specific optimization. The tools then can be used for modular tagging with such tools as FPs, degrons, and APEX2. Transgenic systems and recombinases also will enable the design of drivers for binary systems (e.g., GAL4/UAS, split GFP systems). Both general tool development that can be shared among species and species-specific optimization should be encouraged.

D. *C. elegans* and Disease Research

Suppressor and enhancer genetics are straightforward and powerful in *C. elegans,* and such approaches are unavailable in human studies. Humanization of pathways in the *C. elegans* genome represents an unexplored strategy. Disease alleles can be introduced into the nematode; the resulting metabolic, cellular, developmental, and behavioral effects can be characterized in detail for about 50% of the genes. These phenotypes can be suppressed or enhanced in forward genetic screens and drug screens. The conclusions from such studies would then be more directly applicable to human health.

As genetic manipulations and screens proliferate, researchers will be faced with a deficit in phenotyping tools. Systematic links between genomics and phenomics—from genes to transcripts to proteins to metabolites—must be established. This effort will require multi-omics modeling to build an atlas of cells; transcriptomes, proteomes, and metabolomes from specific cell types and organs; and the whole organism. These efforts are needed to understand genotype-to-phenotype and phenotype-to-disease connections. Databases that integrate information from multiple organisms are needed to share and integrate multi-omics information.

Development of autonomic platforms will facilitate high-speed, high-throughput screens; provide unbiased, quantitative outcomes; and ensure high reproducibility. These platforms would compare organellar and cellular phenotypes in wild-type controls versus mutants. This effort will require cross-disciplinary collaboration among scientists in basic biology and human diseases, chemists for drug screen design and optimization, electronical engineers for high-throughput device building, and data scientists for data analysis.

E. *Drosophila* Methods and Resources

- ***Split protein systems*.** Splitting proteins to create two inactive moieties that reconstitute *in vivo* when expressed in the same or adjacent cells allows powerful new experimental manipulations. This technique has been demonstrated elegantly with the split-GAL4 and split-GFP systems. These strategies facilitate numerous measurements, including FP labeling, measurement of calcium levels in individual cells, and assessment of synaptic connections. Developing new split-protein tools will be challenging. Promising new strategies include intein tagging, which relies on self-excision followed by protein ligation to produce a functional protein. Proof-of-principle data have been published, but current systems are suboptimal and further development of the technology is required. Because hundreds of intein pairs can work in many experimental systems, investment in this area could create a general method to expand the application of split proteins at proteome-wide scale. These methods could be expanded to vertebrates.
- ***GPCR signaling discovery*.** G-protein-coupled receptors (GPCRs) are a large class of cell membrane receptors that mediate sensing of diverse extracellular signals (e.g., hormones, ions, neurotransmitters, metabolites, light). They are used in all eukaryotes, and in humans they constitute the largest class of targets of therapeutic drugs. Recent research has shown that some GPCRs can be tagged with FPs in specific cytoplasmic domains to monitor ligand-binding and receptor activation by changes in fluorescence. Because GPCRs underlie signaling in the nervous system, developmental processes, and the immune response, biosensors to monitor their activity in real time with minimal invasiveness would provide broad applications. A library of GPCR-FPs should be created for use in all MOs, and corresponding lines should be created for targeted expression of these biosensors in flies and worms.
- ***Protein binding tools for visualizing proteins and functional analysis*.** Determining the localization of proteins *in vivo* is paramount to understanding their biological functions. Two main strategies are to (1) generate antibodies or (2) tag the endogenous protein with an epitope or FP. A highly limited number of antibodies are available to the invertebrate community, and standard production approaches are costly and unreliable. In contrast to typical antibodies, nanobodies produced by llamas or other camelids are only ~15 kDa (encoded by ~400 nucleotides of DNA). The small size and good binding properties of nanobodies make them useful for tagging-based approaches; recently, nanobodies that recognized peptide tags have been isolated. Because they are genetically encodable, nanobodies can be fused with functional domains, expressed by a transgene, and used to determine protein localization, degrade a protein (i.e., deGradFP system), re-localize a protein, and trap a protein in the extracellular space *in vivo* in a cell-specific manner. These applications recently have been demonstrated, but this promising technology has not yet achieved wide use. Nanobody libraries can be generated synthetically or purified from llamas, expressed in phage or yeast, and screened against specific antigens. Nanobody libraries should be created for general protein-tagging epitopes and specific invertebrate antigens. The nanobodies generated and the screening platform can be applied in flies and worms to facilitate a wealth of studies. The creation of both nanobody resources for protein tagging (e.g., tags, nanobody fusions, stocks) and nanobodies targeting specific antigens are proposed.
- ***Imaging at subcellular resolution and labeling of interacting proteins: Proximity labeling.*** Biological processes occur in organelles, and protein functions correlate with subcellular localization. Understanding how cellular structures underlie specialized functions requires comprehensive identification of proteins within spatially defined cellular domains in their native environments, and identification of interacting proteins is key to elucidating mechanisms underlying such complex cellular processes as signal transduction, in which transient interactions propagate signals from one protein to the next, relaying information. Mass spectrometry techniques have been used primarily to characterize the proteomes of organelles and to identify

protein interactors by affinity pull-down or following crosslinking. The most common approach to characterizing organelle proteomes and identifying protein interactors or complexes is affinity pull-down or crosslinking followed by mass spectrometry. Purification of organelles or complexes, however, is sometimes impossible, often difficult, and complicated by both false-positive and false-negative discovery (e.g., when an organelle or complex is disrupted during purification). Additionally, a variety of discrete, but functionally relevant, cellular regions cannot be purified, and transient or weak interactions can be lost during purification. These issues have limited researchers' abilities to interrogate the proteome in its native environment. In recent years, proximity labeling methods have emerged that address many of these issues, providing unprecedented tools for mapping protein interactomes and subcellular proteomes in their native environments and in various conditions. These include enzymatic methods based on peroxidases (e.g., horseradish peroxidase), biotin ligases (e.g., APEX, BirA), and pupylation ligase (e.g., Pup). Resources that exploit these methods *in vivo* will provide the community powerful tools to probe cell organization in wild-type and perturbed conditions.

F. Integration of Data Across Scales and Methodologies

The wealth of data from screens often surpasses the analysis capacity and research focus of individual laboratories but could facilitate additional discoveries if integrated and made publicly accessible. By combining single-cell transcriptome profiling, electron microscopy atlases, and light-level imaging of genetically targeted neurons in the fly brain, new biological insights likely will emerge. The ability to compare data from different individual animals, generated at different scales, by different methods, and in different laboratories would be powerful but requires an infrastructure for sharing (e.g., a common nomenclature, software platforms, data organizational infrastructure). Support for centralized databases and the personnel who manage them is critical.

G. Online Resources for Data Mining and Integration

FlyBase, WormBase, and other databases serve as essential meta-databases and repositories for large data sets. Sophisticated algorithms allow experts to mine these data for enrichment analysis, cross-species comparisons, and reagent design. Challenges include navigation of meta-databases and data repositories, integration of information from different sources, and access to command-line and other expert applications. Thus, a need exists for development and maintenance of user-friendly online resources that facilitate data mining, integration, and other functions based on existing and new data and algorithms. These resources are the product of collaboration among MO researchers and other experts, and they help researchers efficiently and effectively make use of MO data for development of new hypotheses and other applications in MO and human studies. User-friendliness and accessibility are valued highly by users. New availability of large data sets (e.g., single-cell RNA sequencing data) will further increase the need for this type of online resource. Support of development and maintenance of these resources is needed.

H. High-Resolution Microscopy

Over the past several years, cutting-edge high-resolution microscopy techniques have been developed, but the high equipment cost and specialized skills required to construct and operate the instruments make the technology inaccessible to 99% of research laboratories. Expanding access will facilitate a wide range of biological studies. These techniques include whole-animal functional and developmental imaging with isotropic spatial resolution, whole-brain imaging with molecular contrast and nanoscale resolution, and scanning electron microscopy for large volume 3D imaging. Creation of regional imaging centers would provide researchers across the United States with access to these advanced technologies.

I. Validation

Studies in worms and flies have led to the discovery of genes, proteins, and pathways that are evolutionarily conserved and play major roles in human diseases**.** The elucidation of developmental pathways by Nobel laureates Drs. Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric F. Wieschaus (e.g., Notch, Wingless, Hedgehog) have played a seminal role in cancer biology and the discovery of rare human diseases. Research on cell death by Nobel laureates Drs. Sydney Brenner, H. Robert Horvitz, and John E. Sulston in worms paved the way for many studies in humans impinging on development, cancer, and other diseases. Other Nobel Prize–winning contributions from flies and worms have included understanding inheritance, olfaction, immunity, and circadian rhythms, as well as the discovery of RNA interference (RNAi).

Research using invertebrate MOs has revealed new, conserved biological processes and mechanisms. About 70% of fly genes have human orthologs; by replacing fly genes with human orthologs, the function of human variants that may cause disease can be studied. In the past 4 years, studies in flies have played an important role in the discovery of more than 30 rare human diseases and led to the discovery of new mechanisms of disease. Moreover, the ability to test humanized flies for U.S. Food and Drug Administration–approved drugs has led to application of these drugs in children for at least three rare diseases.

Also of note is that flies and worms have made unique contributions to understanding the mechanistic underpinnings of human diseases. Flies and worms are closely related to invertebrates with significant human health impact, such as mosquito vectors of disease—which cause millions of deaths per year—and parasitic nematodes. Experimental approaches and knowledge gained in the models inform studies in these species. Lastly, many of the technologies developed in invertebrates are being used in vertebrates (e.g., RNAi, transposon tagging, T2A-GAL4). Validation of invertebrate models will improve the selection and refinement of these systems and continue to lead discovery of new processes that affect humans in almost all areas of biology.

Summary and Suggestions

Worms and flies will continue to play seminal roles in the discovery of new players in human biology and diseases, the elucidation of complex conserved pathways, and the discovery of therapeutic drugs. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- Stock centers
- Molecular tagging of all genes in worms and flies using CRISPR
- Systematic links between genome science and phenomics (i.e., from genes to transcripts to proteins to metabolites)
- Technology to produce split proteins based on intein technology
- Library of GPCR fluorescent proteins for use in all MOs
- Nanobody libraries for visualizing proteins and their functional analysis
- Interrogation of the proteome in its native environment based on proximity labeling to provide tools for mapping protein interactomes and subcellular proteomes in their native environments
- Combination of single-cell transcriptome profiling, electron microscopy atlases, and light-level imaging of genetically targeted neurons

- Model organism databases and centralized databases to mine data from other databases
- Four to six central microscopy facilities for isotropic spatial resolution, whole-brain imaging with molecular contrast and nanoscale resolution, and scanning electron microscopy for large-volume 3D imaging

Chair Summary of Session II on Validation of Zebrafish Models for Preclinical Research

Rebecca Burdine, Ph.D., Princeton University
William Talbot, Ph.D., Stanford University

The participants of Session II discussed and proposed resources and technologies for the validation of zebrafish models in biomedical research for ORIP and the NIH.

A. Zebrafish Models

Since the pioneering studies of George Streisinger and his colleagues 40 years ago, the zebrafish has emerged as a premier vertebrate model system. Forward and reverse genetic approaches have generated thousands of mutations in genes with functions conserved among all vertebrates. These genetic studies in zebrafish have led to key advances in understanding vertebrate development, physiology, and behavior. In addition, many zebrafish mutants represent important models for human disease. The Zebrafish Information Network (ZFIN) lists more than 7,800 publications that relate to human diseases, ranging from rare Mendelian disorders to cancer and infectious diseases. The transparent, experimentally accessible zebrafish embryo allows exquisite understanding of gene function at the cellular level, and many studies have demonstrated the power of combined genetic and cellular approaches in zebrafish to study functions of vertebrate genes and gene networks.

B. Zebrafish Information Network and Zebrafish International Resource Center

The discussants universally advocated strong, continued support for ZFIN and the Zebrafish International Resource Center (ZIRC), which maintain and distribute thousands of zebrafish lines. These resources are critical to the entire field. Their importance will grow as the use of the zebrafish increases, new collections of fish lines are made, and new types of data emerge and need to be integrated into the databases (e.g., -omics, high-resolution whole-animal imaging).

C. Genome Engineering

The zebrafish model system has made many key contributions to research on vertebrate gene function and the genetic basis of human disease. CRISPR-Cas9 strategies have been widely applied to knock out zebrafish genes by introduction of frameshift mutations. Recent work also highlights the potential to humanize the zebrafish genome, which could impart a significant impact on the investigation of the functions of specific human sequence variants in many diseases. Presently, many examples of different mutations in the same human gene causing different phenotypes exist, and an enormous number of human sequence variants have been implicated in human disease but have not yet been tested functionally. Validated animal models are available only in a few cases. Many zebrafish mutants display phenotypes that are extremely similar to humans with mutations in the orthologous gene. Even when orthologous zebrafish and human mutations cause different phenotypes, fundamental similarities in molecular pathways make zebrafish mutants potentially powerful models to understand the function of the gene and investigate possible therapies.

The development and streamlining of promising technologies to introduce precise sequence variants into the zebrafish genome (e.g., GeneWeld, CRISPR-mediated integration cassette) is a high priority. Human disease research would benefit from projects to generate zebrafish models harboring high-priority human sequence variants. In addition, efforts should be supported to introduce precise, versatile tags, (e.g., T2A-GAL4, recombinase target sequences) into many zebrafish genes. For example, collections of T2A-GAL4 insertions would support not only the functional analysis of particular variants, but also an enormous range of *in vivo* cell biological studies (e.g., protein localization, activity, binding complexes) using powerful approaches whose application has been demonstrated in zebrafish and invertebrate systems.

Another priority is to greatly increase the number of cell type–specific regulatory elements for conditional loss- and gain-of-function studies, *in vivo* imaging of cells disrupted in zebrafish disease models, and cell lineage tracing (e.g., single-cell genome editing of synthetic target arrays for lineage tracing).

D. Screening Small Molecules for Therapeutic Activity

Developing zebrafish are small and easily accessible, and researchers have identified small molecules with potent biological activities in numerous studies simply by screening libraries of compounds added to the water. This approach has yielded multiple molecules now being evaluated in clinical trials, and drug screens with zebrafish mutants are a potentially powerful approach to identify new therapeutic compounds. Such screens could be accelerated by screening centers to provide access to (1) chemical libraries, (2) expertise in high-throughput assay development, and (3) expertise in pharmacology.

E. Supporting Technology Development, Novel Genetic Screens, and New Interdisciplinary Approaches to Disease

Although the zebrafish has been established as a powerful model system for many diseases, the discussants recognize that this model's impact would be increased further by new mechanisms to support development of new technologies, new phenotypic assays, and new genetic screens in understudied areas (e.g., adult phenotypes, enhancer and suppressor screens). Disease modeling and other preclinical studies could be accelerated by mechanisms to support and encourage interdisciplinary collaborations among model organism researchers, human geneticists, and clinicians.

F. Nanobody and Antibody Resources

Despite the power of the zebrafish as a system for *in vivo* cell biology, antibodies are available for only a few zebrafish proteins. In light of recent advances in screening for nanobodies and the use of nanobodies in a wide variety of imaging and functional studies, it would be valuable to establish a resource to generate nanobodies to zebrafish proteins.

Summary and Suggestions

Zebrafish are powerful model organisms with well-developed forward and reverse genetic methods that offer novel applications for disease study and drug discovery. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- ZFIN and ZIRC, which are vital resources for the community
- Technologies to humanize the zebrafish genome and knock versatile tags into zebrafish genes
- Centers to exploit fully the potential of chemical screens, genome editing, and imaging in zebrafish to generate disease models and pursue therapies. There would be merit in a "hub-and-spoke" structure, in which the "hub" developed specific expertise (e.g., chemical screening, genome editing, imaging) that was available to support specific "spoke" projects
- Genetic screens and new, high-throughput phenotyping technologies
- New mechanisms to encourage collaborations among clinicians and scientists with expertise in zebrafish and other model organisms
- Nanobodies to many zebrafish proteins implicated in human disease

Chair Summary of Session III on Validation of Mouse Models for Preclinical Research

Cathleen Lutz, Ph.D., The Jackson Laboratory
Douglas C. Wallace, Ph.D., Children's Hospital of Philadelphia and University of Pennsylvania

The participants of Session III discussed and proposed resources and technologies for the validation of mouse models in biomedical research for ORIP and the NIH.

A. Advantages and Challenges of Mouse Models

Although invertebrate and lower vertebrate models of human disease have advantages of short lifespan, low cost, and high numbers of offspring, they lack the critical human physiological property of being endotherms. Large animal and nonhuman primate models have the advantage of increased anatomical and immunological similarities to humans, but they are genetically heterogeneous, produce few offspring, and exhibit a long lifespan. For these reasons, the mouse, which falls neatly between these two extremes, is likely to remain an experimental mainstay for biomedical research for the foreseeable future.

The mouse is an endotherm, has a tractable life span of approximately 2 years, has a genome defined at the nucleotide level, and—with the advent of CRISPR-Cas9 and other gene editing technologies—is relatively amenable to precise genetic manipulation. Although mouse models of human disease are well established, complexities in working with the mouse can lead to inconsistent results if overlooked. The objective of this session was to identify potential areas of uncontrolled variation, assess how these areas might complicate results, and discuss recent approaches to mitigate uncontrolled variability.

B. Genetic Approaches for Mouse Models

Two major genetic approaches—use of inbred strains and use of outbred (i.e., wild-type) strains—are applied in mouse model research. The most commonly used inbred strains originated from three main sources: (1) Abbie Lathrop–derived inbred mice refined by Earnest Castle and Clarence Cook Little, which ultimately led to the inbred strains maintained by The Jackson Laboratory; (2) Swiss mice; and (3) Asian (i.e., Chinese and Japanese) mice. Inbred mouse strains have several advantages, including genetic homogeneity, reproducible and desirable phenotypes, genetic characterization (i.e., often to the nucleotide level), and a passive demeanor for ease of handling in many cases.

The genetic homogeneity of the inbred strains facilitates genetic manipulation via homologous recombination and CRISPR-Cas9 gene alteration. Directed mitochondrial DNA mutagenesis also is possible (e.g., using a transcription activator-like effector nuclease–directed DNA double-stranded cytidine deaminase). Furthermore, inbred strains have given rise to recombinant inbred strains, which have been powerful tools for identifying genetic modifier loci. Disadvantages of inbred strains include their genetic homogeneity, which is not a true model for the highly outbred human population. Likewise, prolonged inbreeding can result in the acquisition of additional mutations, which result in phenotypic differences between sublines. Hence, careful attention to strain background is essential to producing consistent results.

Outbred strains better approximate the heterozygosity of humans and can be more physically robust than inbred strains. Laboratory breeding of an outbred or wild-type mouse strain, however, uses a limited number of breeding pairs and thus will result in genetic drift and the progressive loss of heterozygosity. Generation of consistent heterozygous lines by crossing set combinations of inbred strains gives genetic consistency. This approach, however, requires regeneration of the experimental animals from the inbred strains for every experiment.

C. The Mouse as a Model for Human Disease

In some cases, mice can be used directly as models for human disease. For example, a mouse dysferlin gene mutation (L1341P) impairs membrane trafficking and leads to a muscle phenotype consistent with dysferlinopathy (i.e., dysferlin-based muscular dystrophy) in humans. This muscle defect can be ameliorated with a chemical chaperone, which facilitates the appropriate muscle cellular localization of the mutant polypeptide. In contrast, disease-associated gene mutations can be introduced into mice, and recombinant inbred strains can be genetically characterized; this approach has been used for models of Alzheimer's disease.

Although these studies show the strength of the mouse as a model for certain human diseases, known human gene mutations can fail to obtain the equivalent phenotype in mice. One classic example is Duchenne muscular dystrophy (DMD), which is caused by the inactivation of the dystrophin gene on the X chromosome. In human males, inactivation of the dystrophin locus results in the progressive loss of muscle and cardiac function and, ultimately, death. In contrast, the mdx mouse (i.e., a popular model for DMD) is non-lethal. This discrepancy can be rectified by introducing into the mouse a mutation in the RNA component of telomerase, which results in the recapitulation of the human phenotype. These findings suggest that human muscle satellite stem cells are lost through apoptosis because human cells have shorter telomeres than mice.

As more information has become available about the differences between mouse and human physiology and molecular biology, "humanization" of the mouse through genetic manipulation has become possible. Humanized models can be created by ablating the mouse immune system to support engraftment with human hematopoietic and immune cells and have many biological applications (e.g., SARS-CoV-2, HIV).

D. Environment and the Microbiome as Sources of Mouse Phenotypic Variation

Many human phenotypes reflect the interaction between the individual's genotype and associated environmental challenges. Presently, mouse housing is highly standardized and maintained meticulously free of significant mouse bacterial and viral pathogens. Thus, the environment of the laboratory mouse is not a model for the human environment. In fact, the immune system of the laboratory mouse is thought to be "immature," with pet-store mice producing more memory T cells than laboratory mice.

Moreover, differences in the gut microbiomes of mice of different origin could be a factor in the variability of phenotypes. When pups are cross-fostered with mothers of different genotypes and gut microbiota, the initial gut microbiota of the pup reflects that of the foster mother. Over time, however, the pup gut microbiota drifts back toward that found in mice with the pup's genotype. Because inbred strains maintained for long periods in separate breeding facilities can diverge genetically, differences in the gut microbiota of mice from different vendors could reflect genotypic differences.

Summary and Suggestions

Mouse models offer numerous benefits for biomedical research but present significant challenges for investigators. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- Consistency in sourcing and reporting of inbred mouse strains
- Monitoring of genetic drift in inbred strains via nuclear and mitochondrial genomic sequencing
- Confirmation that the mouse genetic and metabolic systems for a trait of interest are shared between mice and humans

- Consideration of whether the experimental environment approximates that of the human trait of interest (e.g., maturity of the immune system)
- Use of humanized mice in areas where the laboratory mouse is divergent from the human condition (e.g., the immune system, amyloid beta precursor protein sequence, viral infection) to better approximate the human condition
- Storage of mouse fecal pellets for gut microbiome analysis to assess the influence of the gut microbiome on experimental differences
- Confirmation that models for diseases of aging are relevant to the human condition

Chair Summary of Session IV on Validation of Large Animal Models for Preclinical Research

Susan Sanchez, Ph.D., The University of Georgia
Jill Weimer, Ph.D., Sanford Research

The participants of Session IV discussed and proposed resources and technologies for the validation of large animal models in biomedical research for ORIP and the NIH.

A. Large Animal Models in Research

Advantages of large animal models include their phenotypic accuracy for certain diseases and similarity to humans in genetics, anatomy, size, metabolism, and physiology (i.e., relative to mice and other organisms commonly used in research). Certain large animal models mirror human physiology, development, and infectious disease behavior. Large animal models also present unique translational opportunities for developing and testing diagnostic tools and approaches that can be used in humans (e.g., medical imaging, biomarker platforms). Disadvantages associated with various large animal models include increased requirements for time (i.e., longer life cycle), space, technical expertise, and upfront cost. The disadvantages associated with large animal models consequently affect rigor and reproducibility, because fewer animals can be obtained for a single study. In addition, public perception of research using companion or food-source animals is a concern, and historical and comparison data for these studies are lacking.

B. Considerations for Model Selection

Certain criteria that can affect the study design should be considered when selecting models: (1) availability (i.e., for naturally occurring or genetically modified models); (2) ability to replicate the human phenotype for the condition of interest; (3) physiological similarities to humans; (4) size (i.e., in terms of similarity to humans and ability to perform certain procedures); (5) genome accuracy, adequacy, ability to be altered, and similarity to humans; (6) availability of physiologic data on the model; and (7) acceptability of the model with regard to treatment approval. A major limitation of large animal models is their size and the need for appropriate facilities.

C. Standardized Approaches for Imaging

Developing standardized approaches for collecting medical images and processing data captured through imaging could lay the foundation for major advances in biomedical science. Challenges of conducting imaging studies with human subjects are significant and include (1) diversity of disease types, stages, treatment strategies, and comorbidities; (2) recruitment, retention, and scheduling; (3) limitations on frequency of imaging; and (4) biospecimen access. Obtaining sufficient samples and accessing pediatric populations also are challenging in human studies.

Large animal models, such as pigs, offer an opportunity to bridge the gap between small animal models and human subjects for testing translational methodologies. Pig models allow investigators to optimize acquisition parameters for diagnosis and treatment monitoring, perform cross-modality comparisons, examine disease etiology, and conduct well-controlled treatment intervention studies. Imaging in pig models allows investigators to identify internal disease phenotypes noninvasively, compare disease presentation to its presentation in humans, and obtain longitudinal data on disease progression and treatment outcomes using a smaller cohort. Needs for the improvement of imaging in large animal models include (1) ready onsite access to medical imaging equipment; (2) additional training opportunities for scientists to decrease the regulatory, logistical, and technical burden; and (3) core centers for large animal imaging.

D. Functional Connectivity Studies

In addition to their capabilities for imaging studies, pigs are advantageous for neurological research because they possess a large, gyrencephalic brain with a proportion of white matter only slightly less than that of humans. The lack of functional connectivity testing in animal models that are similar to humans in anatomy and physiology represents a major reason for the failure of many stroke treatments in clinical trials. Needs for validation of a functional pig stroke model include (1) a consortium for conducting functional magnetic resonance imaging (MRI) studies of the pig brain to identify functional networks that could be examined for changes after brain injury; (2) validated functional behavior tests for pig models, including standard sensitive testing equipment; and (3) development and standardization of novel methods to measure gait and motor function, which are critical for measurements of stroke outcomes and determination of the effectiveness of new therapies.

E. Naturally Occurring Disease Models

Naturally occurring disease models—such as chronic pain and dystrophinopathies—presented by companion animals (e.g., dogs, cats) can reflect the complex genetic, environmental, temporal, and physiological influences present in humans.

Depending on the specific pain disease state, chronic pain models are common and accessible at veterinary colleges and referral or primary practices. Valid measures of gait and limb use (e.g., activity patterns, quality of movement, somatosensory processing) have been developed for osteoarthritis in dogs presenting clinical disease. Measures of sleep, cognitive function, and affective domains in animals are currently under development and need to be validated. Needs for improvement of the validation of companion animal models for chronic pain include (1) access to species-specific molecular reagents and expertise, (2) improved annotation of canine and feline genome and immune systems, and (3) funding support to optimize the collection of highly phenotyped tissues and establish tissue repositories.

Additionally, dogs exhibit naturally occurring dystrophinopathies and show progressive disease akin to that of Duchenne muscular dystrophy (DMD) patients. Accordingly, canine models for DMD are useful for studies of pathogenesis and preclinical therapy development. Needs for improvement of the validation of canine models for DMD include (1) a centralized, federally funded location to breed lineages prone to this disease and (2) standardization of methods, equipment, functional outcome measures, and reagents.

Summary and Suggestions

Predictive, face, and construct validity must be established for large animal models used in research. Dissemination of information about efforts to validate the models regarding their ability to accurately reflect human conditions (i.e., face validity) is crucial. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- A “Rosetta Stone” of animal models (i.e., standardization of language, definitions, and required validation data) for vertical integration (e.g., collaborative projects, validation)
- Tissue banks for tissue and sample characterization (e.g., genomics, genetic manipulations)
- Phenomics for high-throughput phenotypic characterization (e.g., informatics, AI, big data, storage availability)
- Expanded imaging capabilities (e.g., computed tomography, MRI) for vertical integration (e.g., collaborative projects, informatics) to (1) improve the currently limited access to adequate

facilities, (2) address the current limitations of MRI atlases, and (3) perform MRI software analysis that is specific to large species and standardized molecular sequences

- Standardization of methodology and reporting for vertical integration (e.g., collaborative projects)
- Species-specific molecular reagents for vertical integration (e.g., collaborative projects)
- Naturally occurring models for vertical integration (e.g., collaborative projects, validation)
- Training of veterinarians and future researchers in the complexities of using large animal models for research

Chair Summary of Session V on Validation of Non-Mouse Models for Preclinical Research

Mary Dickinson, Ph.D., Baylor College of Medicine
Kent Lloyd, D.V.M., Ph.D., University of California, Davis

The participants of Session V discussed and proposed the following resources and technologies for the validation of non-mouse models in biomedical research for ORIP and the NIH.

A. Utility and Versatility of Non-Mouse Models

Non-mouse models highlighted in the discussion included rabbits, naked mole rats, guinea pigs, ferrets, rats, and hamsters. This breadth of species is reflected in their versatility and utility beyond more traditional animals (e.g., mice) for modeling the pathophysiology of human diseases at relatively low cost (i.e., compared to larger animal models and human challenge models) to fill key knowledge gaps. Despite these important advantages, these species also have specific limitations that must be addressed to enable integration between different models. For example, each animal species has unique traits and characteristics that inform their physiological and cellular applicability as research models. Nevertheless, data variation using different and unique species can be challenging for investigators to interpret appropriately. To further enhance their utility and versatility as research models, several investments are needed, including (1) resources to support the generation and distribution of non-mouse models, (2) molecular tools and reagents (e.g., specific antibodies) for model validation, (3) support for genome sequencing and annotation, (4) increased support services (e.g., infrastructure, expertise, technologies) for investigators using these models, (5) support for model standardization, and (6) facilitation of collaborative efforts.

B. Rabbit Models

Rabbits have long been used for biomedical research, although their use has declined over time. Rabbit models have particular strengths in certain areas (e.g., muscular and cardiovascular disorders). Mutant rabbit models would enable mechanistic studies and the development of preclinical models for treatment studies. Rabbit models offer several advantages: physiological similarity to humans, previous successful use in translational studies, large size, short gestation period and high proliferation, relative low cost, and ease of genetic manipulation. Challenges associated with rabbit models include limited resources for model creation, high costs for breeding and maintenance, incomplete genomic DNA sequence coverage and gene annotations, and scarce -omics data (i.e., compared to human and mouse data). Creation of a knockout rabbit consortium would (1) generate and supply genetic rabbit models; (2) phenotype genetic lines; (3) provide training on experimental handling, common surgeries, and reagent administration; (4) annotate the rabbit genome and enhance the coverage and accuracy of current genome sequences; and (5) facilitate collaborations between academia and industry. Given the relatively high cost of this model, approaches that enhance efficiency and reproducibility to enable smaller sample sizes are highly valuable.

C. Naked Mole Rat Models

Naked mole rats have gained public attention for their considerable longevity, which is about six times greater than their predicted lifespan (i.e., based on body size). Additionally, naked mole rats display several unique characteristics: (1) hypoxia and hypercapnia tolerance; (2) imperviousness to acid burn and other painful stimuli; (3) hypo-functioning pain signaling associated with osteoarthritis; (4) unusual immune cell populations; (5) resistance to cancer and cardiovascular diseases; (6) resistance to plaques, tangles, and neurodegeneration associated with beta-amyloid and tau in the brain; and (7) abrogated aging. Unlike other mammals, sexual maturity and fecundity increases the chances of life expectancy. Despite the high energetic costs of continuous reproduction, breeding females live longer than subordinates in the colony. Additionally, breeding females do not undergo menopause or exhibit reduced

fertility with age. Furthermore, this species appears to resist common aging phenotypes (e.g., reduced bone mineral density, reduced cardiovascular health) and ultraviolet-induced skin tumorigenesis. Mice and naked mole rats exhibit different physiological responses to ultraviolet exposure. Naked mole rat cells can be transformed by lentiviral vectors with oncogenes, providing a novel resource for assessing mechanisms of *in vivo* cancer resistance. Resources needed to firmly establish the utility of naked mole rat models for biomedical research include public resources or services for investigators, species-specific antibodies, and molecular and genomic tools. For example, many advanced technologies (e.g., CRISPR, RNA sequencing) are unavailable for naked mole rats, and the creation of transgenic mole rats remains a challenge for investigators. Advancements in high-throughput phenotyping (e.g., metabolic chambers, cell painting and imaging, vocalization analyses through machine learning) represent new opportunities in this area.

D. Guinea Pig Models

Guinea pigs are particularly useful as models for assessing infectious disease pathogenesis and treatment efficacy. Historically, they have been used for the effective analysis of bacterial infections (e.g., tuberculosis, diphtheria). Although their immune system has not been studied deeply, some studies have indicated strong similarities with humans. Efforts toward validating guinea pigs as models for human disease have included the following: (1) objective, rather than subjective, disease scoring criteria; (2) technical adaptations resulting in disease that closely mimics human disease; and (3) overall increased reproducibility. Guinea pig models mimic human disease on multiple levels (e.g., cellular, system) and share infectious disease characteristics with humans. Persistent barriers to further validation of guinea pigs as models of human disease include technical challenges (e.g., target dose, lack of reagents) and scientific limitations (e.g., higher challenge dose, objective scoring parameters, lack of disease spectrum). Furthermore, standardization of disease scoring across challenge models is crucial for validation.

E. Ferret Models

Ferrets have been especially useful models for diseases affecting lung, liver, and pancreas (e.g., cystic fibrosis, pancreatitis), many of which occur spontaneously and mimic the pathogenesis observed in humans. Ferrets also exhibit several advantages for studying viral infections (e.g., influenza viruses, coronaviruses, respiratory syncytial virus) and have been useful for studying the pathogenesis, transmission, and vaccine protection of influenza. Recently, ferrets have been used in COVID-19 studies; viral replication has been shown to occur in the respiratory and gastrointestinal tracts, and airborne transmission between ferrets has been detected, making them a useful model for studying mucosal vaccines or therapeutics aimed at the respiratory mucosa. Challenges for further developing ferrets as models for biomedical research include the “orphan status” of many genomes and reagents (e.g., antibodies) for validation. Mobilization of technologies commonly used in mice (e.g., fate mapping, conditional mutagenesis) to ferrets will aid in model validation.

F. Rat Models

Rats have been widely used for many areas of biomedical research and are second only to the mouse as a scientific model species. For example, the striking homology in neural circuitry shared between humans and rats makes them ideal models for studies of mental illness and neurological diseases. The major limitation requiring validation is the sexual dimorphism of disease pathophysiology. For example, consideration of female rats in experimental designs would provide a better understanding of brain function and help investigators identify drugs that might have been overlooked previously in studies conducted only in male rats.

G. Hamster Models

Although an infrequently used animal species in biomedical research, the hamster has gained particular interest recently because of its susceptibility to infection and disease by SARS-CoV-2. Of all animal species used in research today, hamsters represent the human COVID-19 disease course most effectively. Experimental tools for scientific investigation of cell-mediated immunity in hamsters, however, are lacking and represent a crucial validation need. The hamster model provides a valuable tool to study COVID-19 pathogenesis, therapeutics, and preventive (i.e., vaccine) strategies.

Summary and Suggestions

Non-mouse models can play an essential role in biomedical research when other more traditional and commonly used species are inappropriate or lack sufficient human disease relevance. Because many of these systems have limited utility due to the costs associated with the animals and their care, efficient strategies for resource and data sharing are highly encouraged. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- Resources to support the generation and distribution of non-mouse models
- Molecular tools and reagents (e.g., specific antibodies) for model validation
- Support for genome sequencing and annotation
- Public resources or services for investigators
- Support for model standardization
- Facilitation of collaborative efforts

Chair Summary of Session VI on Validation of Nonhuman Primate Models for Preclinical Research

John H. Morrison, Ph.D., University of California, Davis
David O'Connor, Ph.D., University of Wisconsin

The participants of Session VI discussed and proposed resources and technologies for the validation of nonhuman primate (NHP) models in biomedical research for ORIP and the NIH.

A. NHP Models

NHPs are valuable for developing animal models for disease with high fidelity to the human disease phenotype. Genetic links to disease can be exploited both through naturally occurring mutation-driven phenotypes observed in large populations and through gene editing–based exploitation of known disease-causing mutations in humans. NHP models also are valuable for testing therapeutics but can require special engineering for potency. NHPs have proven particularly useful for modeling neurodegenerative diseases, but validation must be extended to the full range of phenotypes observed in humans. NHP models for testing therapeutics (e.g., vaccine development for COVID-19) have taught researchers how to better prepare for the next pandemic.

B. Naturally Occurring NHP Models

Rhesus macaques display greater genetic variation than humans, including more potential functional variation. Functionally significant genetic variation related to disease also is high in rhesus macaques, and researchers can discover and validate spontaneously occurring models for genetic disease by expanding the genetic and genomic information available for existing NHP colonies. Three approaches have been described for the discovery of new NHP models for genetic disease: forward genetics, reverse genetics, and cross-species validation (i.e., testing hypotheses generated in human genome–wide association studies using NHP models).

C. Physiological Monitoring of Emerging Viral Diseases in NHPs

The University of Pittsburgh's Center for Vaccine Research offers technologies to facilitate exposure of a wide variety of mammalian species to aerosol pathogens and respiratory inductive plethysmography to facilitate accurate determination and adjustment of inhaled dose while measuring respiratory function. Physiological monitoring now is performed using implantable telemetry systems that collect data on temperature, activity, blood pressure, intracranial pressure, cardiac parameters, neurological parameters, electroencephalogram, and biopotential at regular and frequent intervals. The monitoring technology is critical to ensuring the reproducibility of NHP studies and offers the advantage of continuous remote collection of data on physiological processes. Advanced technologies enable the collection of more data from each NHP than was possible previously, enhancing detection and validation of complex disease phenotypes and enabling a more sophisticated understanding of complex disease processes.

D. Development of Species-Specific Therapies and Reagents

When modeling therapies for a specific disease in NHPs, investigators must decide which monoclonal antibody reagents are appropriate and should be tested. The therapy should replicate and predict the outcome of therapy in humans. For such diseases as COVID-19, investigators must back-translate therapies that have been tested clinically in humans to animal models. Successful antibody therapy back-translation to animal models depends on several factors (e.g., epitope conservation, relatedness of pathways to human pathways, antigen variability within the colony, antigen density equivalency across cells, antigen tissue distribution comparability to humans). Investigators also must determine whether the therapeutic molecule is specific, has high affinity, and performs similar functions. These challenges can

be overcome by engineering human antibody sequences with higher NHP identity. Back-translation of clinically relevant antibody-based countermeasures will require new tools to aggregate validation information on binding, affinity, function, and immunogenicity in NHPs. The NHP Reagent Resource provides tools for the development of species-specific therapies.

E. NHP Models for Neurodegenerative Diseases

Many neurodegenerative diseases—such as Parkinson's disease (PD), multiple system atrophy (MSA), and Alzheimer's disease (AD)—are associated with aging. Therefore, aged NHP models are needed often but are in short supply. During the session, it was suggested that the National Institute on Aging purchase as many aged (i.e., >20 years old) monkeys as possible, provide support for their housing at the National Primate Research Centers (NPRCs), and make them available for purchase by interested investigators.

NHPs have been critical to the advancement of research on PD and associated therapies (e.g., deep-brain stimulation), with more than 200,000 PD patients receiving relief of cardinal motor deficits. For further study of therapies for PD, scientists need a model of the synucleinopathy that occurs in this disease. Such models have been developed with preformed fibril injections into cynomolgus monkeys to produce the Lewy body pathology associated with PD, and this model displays high fidelity to human PD in its pathology and neurodegenerative processes. A model for MSA has been developed using a vector that affects only the oligodendroglial cells, which are specifically affected in this disease. Additionally, an NHP model for AD has been developed that displays neurofibrillary tangles and neuron loss caused by adeno-associated virus double-mutant tau injections into the entorhinal cortex. Most of these CNS changes can be identified in the NHP models 3 months after exposure, presenting a window of opportunity during which the NHP models can be used to test therapeutics. These models must be validated at the level of *in vivo* imaging, fluid biomarkers, and behavioral decline to match the human disease phenotype as closely as possible.

Gene-edited NHP models for neurologic disorders are expected to be a major focus during the next decade; recently, a gene-edited marmoset model for early-onset AD was developed. This approach entails multiple challenges. For example, although the marmoset is more tractable for such studies than rhesus macaques in many ways, the ability to monitor complex behavior in marmosets is in its early stages and would be facilitated greatly by in-cage voluntary testing approaches, which currently are in development. Social behaviors, sleep disorders, metabolic behaviors, motor functions, and any abnormal behaviors will be assessed across the marmosets' lifetimes using such a system. Infrastructure is needed to support collection and archival of large and expanding data sets (e.g., storage of video data obtained from such systems). Large collaborative teams will be needed to validate such models.

F. NHP Models for COVID-19 and Other Respiratory Infections

NHPs have been used in the study of multiple respiratory infections that occur in humans, including COVID-19 and influenza. The NPRCs, through the Coronavirus Vaccines and Therapeutic Evaluation Network, collaborated to establish harmonized protocols (e.g., procedures, assays) that can be applied uniformly to COVID-19 and other NHP models for respiratory infections, with the goals of improving comparisons of basic studies of disease, supporting evaluation of new vaccines and therapies, and reducing the number of animals needed through shared controls. This effort will enable rapid response to current and future pandemics. NHPs, as the closest model to humans, often are turned to first as the most relevant model of disease.

NHP models have presented several challenges in COVID-19 studies. For example, NHPs often experience a relatively mild respiratory disease compared to humans. Furthermore, more research on COVID-19 in aged NHPs is needed, but insufficient numbers of aged animals are available. Other critical

COVID-19 research areas that will benefit from the use of NHP models include immune correlates, durability of vaccine effects, biotherapeutics, and improved second-generation vaccines.

Lessons learned from COVID-19 research likely will be applicable to the research response during the next pandemic. Influenza continues to pose a risk as a future pandemic virus, and NHP influenza A infections closely mirror symptoms experienced by humans. Additional research opportunities in NHPs include (1) the role of respiratory infections in individuals with chronic lung and immune diseases; (2) the biodistribution and safety of inhaled drugs and antivirals, which cannot be determined in smaller animals; and (3) improving pandemic preparedness by better understanding the pathobiology and immunity to infectious diseases that could emerge to threaten human health in the future.

Summary and Suggestions

Because NHPs carry a high degree of functional genetic variation, researchers can discover and validate spontaneously occurring models for human genetic disease by expanding the genetic and genomic information available for existing NHP colonies. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- NHP resources to effectively anticipate and respond to the next potential infectious disease pandemic
- Expanded breeding colonies at the NPRCs, with improved documentation of pedigrees
- Characterized genetics of all monkeys at the NPRCs, potentially using multiple technologies (e.g., genomics, transcriptomics, proteomics, metabolomics, microbiome analysis) at multiple times throughout the lifespan
- Further development of specialized colonies (e.g., aged monkeys) and increased availability to collaborative teams
- Enhanced capacity for the evaluation of complex phenotypes based on human disease phenotype and encouragement of phenotype experts (i.e., researchers who are not using primates presently)
- Enhanced mechanisms for tissue sharing and digitized primate pathology for remote validation within the NHP community and cross-validation with human and other model system pathologies
- Expanded capacity for breeding and sharing live animal models and support for large, collaborative teams to develop and study gene-edited models
- NPRC involvement in the breeding, maintenance, and veterinary care of gene-edited NHP models with complex phenotypes developed in university laboratories
- Identification and breeding of NHPs with spontaneous disease-causing mutations for disease gene discovery and modeling, as well as expanded sharing of information about those animals
- Development of in-cage voluntary behavioral testing facilities that do not require transport or restraint
- Standardization of NHP brain and section preparation and digitization of section images
- Public communication describing the importance of NHP models

Chair Summary of Session VII on Validation of Non-Zebrafish Aquatic Models for Preclinical Research

Crystal Rogers, Ph.D., University of California, Davis
John Postlethwait, Ph.D., University of Oregon

The participants of Session VII discussed and proposed resources and technologies for the validation of non-zebrafish aquatic models in biomedical research for ORIP and the NIH.

A. Evolutionary Models for Human Disease

Some aquatic animals possess health-promoting capabilities that humans lack, and others express phenotypes that would be pathogenic in humans but are beneficial to these species in their extreme environments. For example, amphibia and some fish can regenerate appendages, functional spinal cords, and hearts after severe injuries. Other species possess potent tumor suppressor genes that become apparent only after outcrossing to closely related species. Many aquatic species have adapted to extreme environments (e.g., perpetually dark caves, constant sub-zero water temperatures, toxic Superfund sites) by evolving physiological and morphological features that mimic metabolic syndrome, profound anemia, bone loss, or what would be inappropriate responses to toxins in humans. The mechanisms of novel abilities—and of compensations for traits that would be pathological in other contexts—can provide insights into molecular, genetic, cellular, and developmental disease etiologies, resilience, and therapies.

B. Genetic and Genomic Resources

The increasing availability of sequenced genomes now makes accessible species that formerly were intractable to genetic investigations. To leverage genetic and genomic opportunities in emerging models, however, continued support for genome resource development is needed. Although the creation of comprehensive panels of CRISPR knockouts or transgenic lines is unnecessary for niche aquatic models, many other communities require access to these and other cutting-edge genetic technologies (e.g., CRISPR-mediated integration cassette [CRIMIC] in flies, single-cell RNA sequencing) to rapidly advance specific research areas that are best studied in aquatic models.

C. Informatic Integration

Genome sequences are useful only if accompanied by appropriate annotation. Accurate cataloging and nomenclature of coding and non-coding genes are essential to achieve vertical integration. Vertical integration will connect genes, conserved non-coding elements, epigenetic marks, and other genetic components of each aquatic model to their orthologs in other aquatic models, terrestrial models, and—in particular—the human genome.

Multispecies informatic sites can more efficiently relate species to one another than single-species sites. The Alliance of Genome Resources (AGR, alliancegenome.org) comprises six model organism databases (i.e., yeast, worm, fly, zebrafish, mouse, rat). The Gene Ontology Consortium (geneontology.org) aims to integrate and distribute organismal data to basic biologists and clinicians. The AGR is unlikely to expand to non-zebrafish aquatic models for preclinical research but provides an opportunity to link aquatic model organism data to human biology more effectively through existing members of the AGR consortium.

Major vertebrate aquatic models occupy two main evolutionary lineages: one that includes most familiar fish (i.e., ray-finned vertebrates) and another that includes amphibians and terrestrial vertebrates (i.e., lobe-finned vertebrates). More basally diverging lineages, such as cartilaginous vertebrates (e.g., sharks, rays) and jawless vertebrates (e.g., lampreys), also provide important models. For amphibians, Xenbase (xenbase.org/entry) collates genomic data, gene expression results, literature, and

other features supporting research on *Xenopus* frogs. Expansion of Xenbase or its underlying structure to the axolotl (*Ambystoma mexicanum*)—which has emerging bioinformatic needs—and potentially other amphibians would help to foster and exploit connectivity among amphibians (e.g., whole-organ regeneration). Connecting amphibian species through a common database would accelerate their validation and vertical integration into preclinical research pipelines.

The Zebrafish Information Network (ZFIN, zfin.org) connects zebrafish gene nomenclature, function, disruption, and expression data to human orthologs and human biology. Expansion of ZFIN to other selected ray-finned fishes would represent a crucial step toward harmonizing results across species. Important candidate species include Mexican tetra cavefish (*Astyanax mexicanus,* which displays metabolic syndrome, behavioral biology, blindness, and other health-related phenotypes), *Xiphophorus* fishes (which display melanomas and genetic modifiers of tumor biology), medaka (*Oryzias latipes,* which contains a unique large genetic reference panel of near-isogenic wild lines to investigate roles of modifier loci), stickleback (*Gasterosteidae,* which can be applied to bone diseases, the microbiome, and toxicology), killifishes (which can be applied to the evolution of aging and resistance to environmental toxins), and spotted gar (*Lepisosteus oculatus*, which is a basally diverging ray-finned vertebrate with a non-duplicated genome that bridges teleost genomes to human biology). The horizontal integration of these fishes to zebrafish and to one another—coupled with the vertical integration of zebrafish to invertebrates (e.g., worms, flies) and tetrapods (e.g., mice, rats, humans) through the AGR—would accelerate the utility of these evolutionary disease models for human biology.

D. Resource Centers

Access to healthy, standardized experimental animals is essential for reproducibility of experimental results across various laboratories. Such animals can be supplied efficiently by dedicated resource centers, such as the National *Xenopus* Resource (mbl.edu/xenopus), *Ambystoma* Genetic Stock Center (ambystoma.uky.edu/genetic-stock-center), *Xiphophorus* Genetic Stock Center (xiphophorus.txstate.edu), Zebrafish International Resource Center (ZIRC, zebrafish.org), and National Resource for *Aplysia* (aplysia.rsmas.miami.edu). Expansion of current stock centers to a few other key species would greatly improve access to healthy and genotypically secure animals. Stock centers are especially important for aquatic model species because large populations and dedicated mating schemes are required to ensure the retention of genetic diversity, but most individual laboratories are unable to retain large stocks because of cost and space constraints. A multispecies stock center could develop expertise on sperm cryopreservation methods to preserve precious stocks and genotypes. The inclusion of additional species to an existing stock center would allow economy of scale and shared personnel. For example, the *Xiphophorus* Genetic Stock Center or ZIRC could add additional species stocks (e.g., cavefish, medaka, stickleback, killifish).

Because several risks exist when maintaining various species in proximity (e.g., agents of infectious disease that are asymptomatic in one species but pathogenic in another), the design and functioning of multispecies stock centers is critical. In addition, species-specific expertise might be needed in some cases. Potential risks, however, can be investigated and managed to provide researchers across the United States access to validated, standardized, healthy research model organisms and thus increase their validity as models for human health and disease.

E. Aquatic Models of Human Disease Conference

Periodic meetings within research communities provide opportunities to share innovative ideas and develop new collaborations. The Aquatic Models of Human Disease Conference (mbl.edu/aqmhd) brings together a long-established community in a meeting held every other year since 2001. The 10th meeting—supported by ORIP, the National Institute of Environmental Health Sciences, and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development—is scheduled to occur in

person on October 7–11, 2021, at the Marine Biological Laboratory, Woods Hole, Massachusetts. Recurring conferences are essential to bring together the aquatic model organism community, introduce young investigators to the field, share best practices, provide training opportunities and workshops, and illuminate emerging opportunities for research.

Summary and Suggestions

Non-zebrafish aquatic models have emerged as strong research organisms to identify and model mechanisms of human disease. This session addressed several aspects of validation, including face, construct, and predictive validity. Although the community of aquatic medical models is diverse with specialized needs, several unifying themes emerged during the session. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- Creation of platforms to support horizontal and vertical integration of information (e.g., genomic, transcriptomic, proteomic, metabolomic, phenomic, anatomical nomenclature) among laboratories and research institutes for increased transparency, collaboration, and expeditious discovery
- Creation and maintenance of annotated genomes (i.e., informatics) for non-zebrafish aquatic models tied to existing members of AGR
- Expansion of current resource and/or stock centers to include additional species, ensuring access to various aquatic models
- Research conferences to bring together participants using nontraditional and unique aquatic models for human disease
- Mechanisms for the creation and validation of such molecular tools as transgenic animals, verified antibodies, and vectors (e.g., similar to CRIMIC in flies) that can be utilized within and across species for further vertical integration and validation

Chair Summary of Session VIII on Technologies, Phenotyping, and Data Science for Animal Models

Keith Cheng, M.D., Ph.D., Penn State College of Medicine
Stephen Ekker, Ph.D., Mayo Clinic

The participants of Session VIII discussed and proposed technologies, phenotyping, and data science for the validation of animal models of human biology and disease supported by ORIP and the NIH.

A. Technological Needs and Opportunities

In prior sessions, needs and opportunities across model systems were discussed; topics included limited resources (e.g., stock centers) and web-based resources for data curation, mining, processing, and integration. Community-building and collaboration tools are needed to stimulate interdisciplinary research. Additional needs include *in vivo* imaging methods to characterize metabolic processes and physiology in real time, genetic tools (e.g., gene tagging for localizing and quantifying gene expression), and genome editing. Antibody resources—in particular nanobody resources—are needed for localization and functional analysis. Imaging services can be used for sophisticated methods in which specialty knowledge is required that extends beyond that of biologists (e.g., lattice light-sheet microscopy at the Janelia Research Campus).

B. Genotype and Phenotype Integration

The research community's focus is gene- and molecule-centric; this approach is attributable to progression from (1) the realization of the existence of genes and molecules to (2) the development of molecular biology, genome sequencing, and computational (e.g., bioinformatic) analysis and, presently, to (3) genome editing and computationally enhanced -omic tools (e.g., single-cell transcriptomics, proteomics, metabolomics). Several principles, however, indicate that genes and molecules alone will not yield an effective understanding of biology and disease: (1) Comprehensive histology-based tissue phenotyping in zebrafish has shown that single-gene deficiencies frequently cause multiple phenotypes across organ systems, (2) different mutations in single genes can cause different phenotypes, (3) mutations in different genes can cause the same phenotype, and (4) human gene–specific deficiencies cause sets of phenotypes that define clinical syndromes. Genes and molecules require spatial context and knowledge of cellular and tissue phenotype to yield the most biologically and clinically relevant understanding of biology or disease.

C. Phenomics

A notable gap in validation has been a relative lack of attention to the systematic study of phenotype (i.e., phenomics). Tissue phenotype is critically important. Because nearly all human diseases are associated with 3D micron-scale changes in cells and their arrangements in tissues (i.e., as detected by histology), tissue phenotype provides a relatively unbiased approach to characterizing and addressing human disease. Histology alone, however, is plagued by (1) its descriptive and subjective nature; (2) the virtual absence of volumetric value; (3) and the current lack of objective, quantitative parameters that can be correlated with genetic and molecular data using data science (e.g., approaches using ML or AI). Computational tissue and organismal phenomics can be employed to bridge this knowledge gap.

Because every human disease is associated with micron-scale changes in cells and tissues—and knowledge of those phenotypes is required to understand the cellular basis of body function, development, aging, disease, and responses to environment (i.e., both natural and anthropogenic)—tissue phenotype is important for computational accessibility. A first-principles approach to tissue phenotyping identifies 3D phenotyping across cell types (i.e., "pan-cellular phenotyping"), millimeter-to-centimeter

fields of view, submicron resolution to score cellular features critical to tissue phenotype, and the ability to cross-reference findings to established histopathology slide scans that have been validated by pathologists and comparative pathologists. The imaging aspects of these requirements have been fulfilled by the development of a form of micro-computed tomography (micro-CT) that is customized for tissue phenotyping at the organismal level. This tool is usable across model organisms, is in early stages of development, and will be greatly enhanced by the development of objective tools for computational phenotyping to remove subjectivity and add biologically and clinically relevant quantitative detail to genetic, chemical, and disease phenomics.

Computational tissue and organismal phenomics will be most useful when integrated with full sets of gene expression patterns across model systems and across full life spans. This goal—although not yet attained for *Drosophila*, aquatic models, or mammals—is potentially attainable using a transgenic stain that would yield gene-specific expression of a metal-binding protein or substrate. Phenomics will be necessary for true deep integration of genetic, disease, and environmental phenomics to accelerate understanding, diagnostics, and drug development. The goal for all advances in molecular imaging and imaging at multiple scales (i.e., from atoms to organisms) is for integration—through computationally accessible parameterization—of normal and diseased cell, tissue, and organismal structures.

D. Deep Integration

A maximal depth of understanding will henceforth come from the integration of the full range of clinical and experimental data (e.g., clinical or model systems), crossing length scales and technologies. The wealth of data across model systems and humans is so abundant and complex that it now is impossible for any single laboratory to analyze and integrate all data that might be relevant to their investigations. Data must be preserved as accessible resources, accompanied by visualization and analytical tools for discovery. Because file sizes are large (i.e., individually and collectively), and the computational demands of analytics and visualization exceed the capabilities of any single server, full integration across model systems, scales, and methods will need to be cloud-based. Integrated teams have begun to explore disease processes across length scales and modes of study, revealing the scientific value of integrated, open systems for data sharing, tool generation, and resource sharing.

E. Community Imaging Resources

Lattice-sheet and other complex fluorescence imaging technologies were discussed in Session I. A need exists for customized micro-CT (i.e., large-scale), 3D tissue and organismal phenotyping, and X-ray histotomography. These technologies originally were developed using synchrotron resources, but instrumentation now can be created for institutionally based histotomography. Synchrotron-based accessibility needs must by coordinated with upgrades to two primary synchrotron sources in the United States (i.e., Argonne National Laboratory and Lawrence Berkeley National Laboratory) over the next several years; these efforts would require a mail-in model by shipping samples for imaging (i.e., with cloud-based accessibility, visualization, and analytics) and cloud-based accessibility to computational and visualization tool expertise. The COVID-19 pandemic currently limits this work to institutionally based instrumentation that could be explored for expansion; this alternative approach has potential for broader availability within 5 years. Collaborative development of computational phenomics will be necessary, inclusive of global involvement.

Summary and Suggestions

Technology, deep phenotyping, deep integration, and data science (e.g., ML, AI) will play new key roles in advancing the use of model systems for understanding human biology and disease. Phenomics will enrich genetic and molecular studies across length scales and enhance discovery and translation for the

improvement of human health. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- Advancement of cell- and tissue-oriented computational phenomic imaging technologies—especially those for comprehensive phenotyping that includes organismal context—emphasizing tools that are applicable across model systems to facilitate cross-validation
- Broadened availability of imaging technology and related tool development to enhance and integrate genetic, disease, and chemical phenomics
- Enhancement of quantitative tools for tissue phenotyping using ML and AI
- Creation of comparative pathology tools for validation of the similarity of tissue phenotypes between human and model system forms of disease, inclusive of linkages between 2D and 3D data
- Creation of mechanisms for adding tissue and organismal context to -omics data (e.g., single-cell transcriptomics, proteomics, metabolomics)
- Enhancement of real-time analysis of metabolic and physiological processes and integration across scales and imaging by requiring sharing of data, analytical tools, and processes
- Creation of cloud-based links between genotype and phenotype across length scales and -omics, (e.g., user-friendly computational workflow systems that implement collaboration and data integration)

Chair Summary of Session IX on Vertical Integration Approaches for Preclinical Research

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The participants of Session IX discussed and proposed strategies for vertical integration of various animal models for human disease for ORIP and the NIH.

A. Vertical Integration of Animal Models for Human Disease

Vertical integration of animal models for human disease requires validation between and across the species of interest. Integration of genetic data often is challenging for researchers. In previous sessions, several key goals and areas of tool development for vertical integration and associated validation were identified: (1) integration of model organism and clinical data to identify mechanisms and potential means of therapy at level of individual patient care, (2) a comprehensive understanding of biological mechanisms, (3) insights into evolutionary biology, and (4) development of approaches with scaling potential to scale to overcome current challenges in biomedical research.

B. Informatic Strategies and Tools

Information strategies necessary for vertical integration include dynamical modeling, which rarely is performed outside membrane biology; network analysis for more robust data sets and experimental support; machine learning and AI for data scaling; and black-box strategies, because most gene functions remain unknown (i.e., phenotype gap). For each of these strategies, computational biologists identified the need for larger volumes of standardized phenotypes (i.e., extant and novel) at the cellular and organismal levels.

Informatic needs for vertical integration include (1) the breakdown of information silos and more centralized resources (e.g., the Alliance of Genome Resources); (2) aggregation, weighting, and display of existing disparate data in formats accessible to end-users (e.g., Model organism Aggregated Resources for Rare Variant ExpLoration [MARRVEL]); (3) prediction of genome function at the individual nucleotide level; (4) prediction of protein function at the individual residue level; (5) prediction of the best model organism for specific biological questions; (6) generation of quantitative multiscale models of biological processes; (7) generation of robust predictive tools to accelerate empiric discovery; and (8) creation of platforms to link multidisciplinary teams capable of solving substantive problems to these problems—including specific clinical problems.

C. Decoding of Genomes, Annotation of Genome Function, and Experimental Tools

Models are needed at the level of alleles or candidate variants in individuals to assess outcomes of variants. These models should consider all alleles of the gene of interest. Generalizable annotation of all locations in the genome enables gain- and loss-of-function**s** and cross-species insights into genome function. Additionally, somatic genetics can be leveraged to accelerate annotation.

Experimental needs for vertical integration include "off-the-shelf" tools for allelic series (e.g., mutants through stock centers); deep mutational scanning programs (e.g., cellular and functional assays, saturation mutagenesis, sequencing); phenotypic assays that span cells through phyla to humans; more efficient and generalizable tools for gene, RNA, and protein expression and localization; broader functional reporter tools (e.g., for subcellular localization); and access to "gold standards" for existing phenotypes (i.e., empiric biological outcomes).

D. Phenotypic Integration and Decoding Biological Circuits

Ontological and experimental approaches to phenotype comparison should be considered for integration, and iterative cycles of empiric data are needed. Additional needs include high volumes of high-content phenotypes to train and validate informatic models; robust molecular, cellular or tissue-level phenotypes that span different species; and defined phenotyping standards. Humans should be considered as a model organism because data integration requires a multidirectional approach.

Decoding biological circuits will require the combination of iterative computation with empiric experimentation, the use of multiscale models (e.g., gene, RNA, protein, metabolite, cell, tissue, organ, organism, population), and the identification of generalizable biological logic (i.e., moving beyond select cases).

E. Shared Structured Data Collection

Requirements for shared structured data collection include formal power estimates and explicit data on reproducibility; data acquisition standards and calibration (e.g., genotyping rigor, data formats, perturbations, phenotypes with possible minimal data sets in specific contexts); shared genotypes, phenotypes, and perturbations across context and species (i.e., phenotyping by design); digital data collection with raw data that include upload date and time stamping (i.e., to avoid error and fraud); and scalable techniques.

F. Programmatic Innovation for Integration and Bidirectional Translation

Promotion of collaborative programs enables testing of best approaches for vertical integration. A need exists, however, for innovative clinical programs within this framework to (1) align new phenotypes in patients with those in model organisms and (2) perform formal testing of mechanistic hypotheses in humans. These platforms will enable the focused efforts of disparate investigators around the completion of prespecified comprehensive data sets (e.g., phenome projects). These changes, however, will require reimagining relationships among patients, academia, government, and industry.

Strategies to foster and optimize bidirectional translation include the development of co-clinical modeling centers with clinical and model organism expertise, efficient access to fundamental expertise in the function of a specific gene or pathway, reimagination of clinical trial designs (e.g., earlier cross-talk between human and model organism expertise), and long-term support for the transition of clinical observations to basic science with close engagement of the model organism community (e.g., co-clinical modeling).

G. Funding and Associated Innovations

Opportunities for funding and innovation include (1) databases and stock centers built into every NIH budget and budgets contingent on delivery of standardized phenotypes and sharing of data and reagents, (2) multidisciplinary centers to test different approaches to vertical integration (e.g., computational, model organism, chemical biology, cell biology, human genetics, clinical expertise), (3) multidisciplinary centers to test the same approaches to vertical integration in different disease areas, and (4) cross-disciplinary education for vertical and horizontal integration.

Summary and Suggestions

The following general questions have been identified for vertical integration: (1) What data are missing? (2) How can the gaps be filled in a directed fashion? (3) Should all NIH-funded model organism experiments require the collection of exome or genome data to leverage all experiments for genome annotation? (4) Which of these efforts should be aligned through mandates across certain types of NIH research? (5) How can efforts be coordinated to ensure ambient vertical integration? (6) What is the ideal unit or use case for ambient vertical integration to occur? (7) How can the best approaches be defined quickly? The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- Infrastructure to change the scale of data collection (e.g., genomes to alleles, individuals to populations), including new tools in genetics, genomics, proteomics, cell biology, cell physiology and new approaches to data collection (e.g., ambient integration to minimize mandates)
- Infrastructure to optimize all data collection and data management across the NIH and beyond, including standard data collection requirements for genotypes, phenotypes, perturbations (i.e., metadata); standard formats and documentation; formal testing of discrete analytic approaches against empiric outcomes; and long-term integrated support for databases and stock centers
- Infrastructure to align data collection around common goals (e.g., decoding genomes, decoding biological circuits, creating discrete platforms and approaches to connect investigators and data)
- Infrastructure to support the development of a skilled workforce and ongoing creative research paradigms (e.g., cross-disciplinary communities, cross-disciplinary education)