The Sustained Impact of Model Organisms—in Genetics and Epigenetics

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Now that DNA sequencing can reveal disease-relevant mutations in humans, it is appropriate to consider the history of genetic research on model organisms, and whether model organisms will continue to play an important role. Our view is that genetic research in model organisms has had an indisputably enormous impact on our understanding of complex biological pathways and their epigenetic regulation, and on the development of tools and approaches that have been crucial for studies of human biology. We submit that model organisms will remain indispensable for interpreting complex genomic data, for testing hypotheses regarding function, and for further study of complex behaviors, to reveal not only novel genetic players, but also the profound impact of epigenetics on phenotype.

MODEL organisms have been instrumental in providing insight into many biological problems and for revealing fundamental mechanisms that underlie disease. These influences and contributions of model organisms are unlikely to diminish. The tools of genetics that are often conceived and developed with model organisms enable the challenges presented by more complex aspects of biology, such as disease and behavior, to be tackled.

Our expertise is with *Drosophila, yeast, and cellular models for biological processes and disease (Warrick et al.* 1998; Lo *et al.* 2001; Auluck and Bonini 2002; Dang *et al.* 2009; Govin *et al.* 2010; McGurk *et al.* 2015; Sen *et al.* 2015). Here, we highlight some of the major impacts of model organism genetics, and what we anticipate are major opportunities in the future.

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The Rise of Model Organism Genetics and the Embrace of Molecular Genetics

Model organism genetics has revealed many fundamental biological processes. This was achieved by applying unbiased genetic screens to reveal genes and their mechanisms of action in processes such as cell cycle control (Nasmyth 2001), basic body plan specification and mechanisms of development (Lewis 1978; Nusslein-Volhard and Wieschaus 1980), and cell death pathways (Ellis and Horvitz 1986; Avery and Horvitz 1987), all of which were recognized with Nobel prizes.

The sequencing of genomes revealed the breadth of the conservation of genes, and of entire pathways. It also brought a desire to apply reverse genetics to query the functional roles of genes. The ability to recognize human disease genes by their homology to genes in model organisms was an impetus to mimic human disease pathologies in simpler experimental systems. Loss-of-function and gain-of-function approaches can reveal the normal function of pathways that are defective in disease. Indeed, the biology of pathways revealed by studying disease mutations is often presented in ways that are difficult to attain through classical loss-of-function approaches. For example, the study of proteins that accumulate in dominant degenerative diseases in humans have provided tremendous insight into the nuances of protein folding, protein degradation, and the proteasome pathway (Balchin *et al.* 2016).

Extending Genetic Approaches from Model Systems

One of the advances with greatest impact has been application of unbiased genetic screens on a broader scale that encompasses all genes of an organism. Such approaches include libraries of RNAs fed to *Caenorhabditis elegans* animals to knock-down every gene involved in a particular process

(Kamath *et al.* 2003; Lee *et al.* 2003), to libraries of gene knockouts and overexpressed genes in yeast (Winzeler *et al.* 1999; Zhu *et al.* 2001), to libraries of shRNA transgenes in *Drosophila* (Ni *et al.* 2011). Such approaches allow unprecedented coverage of the genome, from known factors to newly recognized small RNAs. This approach will undoubtedly continue to have an impact.

Application of unbiased genetic screens to other organisms, and even to cells in culture, where classical genetic approaches cannot be applied, is accelerating (Bassik *et al.* 2013; Mohr *et al.* 2014). More comprehensive and elegant approaches of gene knockdown or gene knockout, and cell manipulation, are leading to "disease in a dish" models that powerfully complement existing *in vivo* studies (Bellin *et al.* 2012; Lancaster and Knoblich 2014). Such approaches enrich, but do not replace, *in vivo* studies, where the nuances of pathway dynamics in intact biological systems repeatedly reveal unexpected and surprising insight. Nearly any organism of interest that can be imported into the laboratory can be developed for study. Examples include mosquito, planaria, fish species, and complex social organisms such as ants (Perrimon *et al.* 2010; Yan *et al.* 2014).

Epigenetic Phenomena Were Initially Recognized and Investigated in Model Organisms

The term epigenetics, and the idea that development proceeds from a single fertilized embryo into innumerable complex cells and tissues, stems from the 1950s (Waddington 1957). It is remarkable that a more precise and modern concept of epigenetics—that heritable changes in phenotype can occur without altering genes—can be traced to earlier observations of variegating eye color in *Drosophila* that changes from generation to generation (Muller 1930). Observations of telomere position effect in yeast underpinned similar seminal ideas that switching between phenotypes occurs without genotypic change (Gottschling *et al.* 1990). Thus there was a slowly dawning realization of genes in three-dimensions, with a past history, and an impact on the future beyond DNA mutations, *i.e.*, epigenetics.

The modern era of chromatin biology and epigenetics was opened by a synthesis of biochemistry and genetics. Indeed, the major chromatin modifying enzymes—histone modifiers, their associated effector proteins, and ATP-dependent remodelers—while requiring crucial biochemical analysis to reveal their activities, were reliant on insightful genetic screens, and structure/function approaches, to unveil the biological context of these activities (Berger *et al.* 1992; Brownell *et al.* 1996). Epigenetics can be considered to be still in its infancy, with the continued emergence of new players (lncRNAs), new states (bivalent promoters), and new histone modifications.

Chromatin has become recognized as not simply a scaffold, but a specific regulator of genes. This goes well beyond transcriptional activation and elongation, capping, and termination of RNA, by extending to every aspect of DNA transactions, including replication, recombination, and regulation of telomere and centromere function. Again, this understanding emerged from a combination of biochemical and genetic approaches. While there is currently a fairly robust understanding of the meaning and impact of histone modifications in the two-dimensional realm of DNA and RNA, recognition is emerging that the three-dimensional structure of chromatin in the nucleus is central to gene expression and its regulation in all biological processes (Dekker and Mirny 2016). Genetics will continue to play a prominent role in revealing the functional impacts of these higher order regulatory features. Overall, the expansion of the reach of epigenetics richly illustrates how integration of biochemistry with modern genomics rests on the shoulders of classical genetic experiments.

Ramping up for the Future

On this foundation of approaches and techniques, what might the future hold?

"Better" models

Whereas initial models for disease and biological processes were developed with gene upregulation, downregulation or misexpression, a more accurate wave of models is emerging in which the endogenous genes are modified *in situ*, for more faithful, although perhaps more subtle, outcomes. The advance of techniques to manipulate the genome *in situ* is widely recognized as a superior way to mimic the endogenous biological situation.

Novel findings from human sequencing studies can be profitably addressed in model systems

We are in the era of human biology as the driver of genetic discovery, thanks to the ease of sequencing the genomes of individuals. Because of the genetic diversity of human populations, sequence data are highly correlative. Sequence relationships with potential biological outcomes must be tested in the rigorous experimental settings provided by model organisms.

Incorporation of more "risk" factors into in vivo disease models

Identification of the genes affected in familial disease is a critical first step to a genetic model for disease. But we have little insight into risk factors for disease. Model organisms enable incorporation of these risk factors—heavy metals, environmental toxins, ageing—for mechanistic insight. Integration of risks will flourish with the application of techniques to incorporate newly identified players and suspected influences; for example, the gut microbiome to physiology, and head injury to neurodegenerative disorders.

Druggable pathways

The recognition that specific genes or pathways can be modulated by small molecules brings these types of reagents to bear on *in vivo* models. For example, defining reagents that

mitigate dominant oncogene activity in an animal model can identify effective therapeutics (Dar *et al.* 2012). This approach also holds the promise of defining targets of a compound (Hughes *et al.* 2000), and of using directed evolution to create the gene activities of interest (Arnold 2015).

Understanding complex traits

Model organism genetics was classically used in single gene studies, but it can be applied effectively to dissect complex traits to reveal how a number of genes contribute to a biological process. It is also possible to reveal risk factors for disease that are identified only as SNPs (Shulman *et al.* 2011): model organism genetics reveals *what can be* a modifier; human genetics informs *what is* a modifier in various human populations.

Epigenetics in disease, behavioral, and transgenerational studies requires rigorous genetics

One of the most impactful insights to emerge from classical genetics is the role of epigenetics in regulating phenotypes. The study of epigenetics promises to reveal new foundational principles of complex traits, including disease, behavior, and transgenerational inheritance. Tantalizing recent discoveries suggest that epigenetics may contribute not only to the maintenance of a cellular/organismal state, but may also drive disease: cancer can be associated with mutations in chromatin regulators (Dawson and Kouzarides 2012). Here, classical genetics has provided important insight into the role of chromatin biology in human disease and complex organismal and social behavior, where profound differences can be attributed to diet and environment, with the role of epigenetics revealed by studies of social organisms (Simola *et al.* 2016). Finally, the role of epigenetics in transgenerational inheritance is now of great medical interest due to diet and other environmental impacts that have the capacity to be carried through the germline. It is challenging to prove inheritance of epigenetic traits, and rigorous genetic studies of model organisms will be necessary to gain deep understanding of these phenomena.

Concluding remarks

Model organisms will remain critical for understanding basic biological pathways and processes as our focus turns toward tackling ever more perplexing and complex processes of behavior and variable risks of disease. Established approaches, and new ones that are sure to be developed, promise to unveil the roles of genes and the epigenome in the state of the organism, and provide explanations of the past and its impact on future generations.

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