Back to the Future: Mutant Hunts Are Still the Way To Go

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ABSTRACT Innumerable breakthroughs in many fundamental areas of biology have come from unbiased screens and selections for mutations, either across the genome or within a gene. However, long-standing hurdles to key elements of mutant hunts (mutagenesis, phenotypic characterization, and linkage of phenotype to genotype) have limited the organisms in which mutant hunts could be used. These hurdles are now being eliminated by an explosion of new technologies. We believe that a renewed emphasis on unbiased mutant hunts, in both existing model systems and in those where genetics is just now becoming feasible, will lead to new seminal discoveries and surprises.

KEYWORDS mutant hunts

YOGI Berra once said, "It's tough to make predictions, especially about the future." We agree. In considering the future of genetics we cannot predict what previously-unknown cellular component will be identified as an element of genetic transmission or what new method will reveal how the brain works. However, we can predict that the future of genetics will be dominated by what has worked so stupendously well in the past: the mutant hunt.

For geneticists working on model organisms, one of the most valuable ways to obtain important and often surprising results has been through unbiased mutant hunts. This is an unparalleled approach to survey a genome to find genes involved in a process, or to survey an individual gene to find alleles that fully reveal its function. Mutant hunts have led to critical discoveries in many fundamental areas in biology, opening new doors into understanding the nature of gene function, how cells divide and regulate gene expression, and how organisms develop and behave. Historically, this approach dates back to Beadle and Tatum (1941), whose work led to the one gene–one enzyme hypothesis, but also led to the concept of using a mutant screen to investigate gene function (Strauss 2016). Some outstanding subsequent examples include the elucidation of the process of eukaryotic cell division (Hartwell *et al.* 1974; Nurse *et al.* 1976), the illumination of circadian rhythms (Konopka and Benzer 1971), and the elucidation of how the metazoan body plan is determined (Lewis 1978; Nusslein-Volhard and Wieschaus 1980).

Mutant hunts make few assumptions. They simply ask a living organism to answer a question, regardless of what the answer might be. As a result, mutant hunts often lead to surprising findings such as the discovery of micro-RNAs, a conserved class of gene not previously known to exist (Ambros and Horvitz 1984; Lee *et al.* 1993; Pasquinelli *et al.* 2000), or the discovery that blue-light receptors in plants are closely related to circadian rhythm proteins in animals (Koornneff *et al.* 1980; Ahmad and Cashmore 1993; Stanewsky *et al.* 1998; Cashmore *et al.* 1999). Mutant hunts have been one of the most powerful ways to lead biologists to look beyond the light under the lamppost.

In some ways, there are striking parallels between mutant hunts in model organisms and human genetics. Human geneticists have performed unbiased screens through the study of individuals with diseases, resulting in the discovery of genes important for human health. Notable cases include the

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identification of BRCA1 (Hall *et al.* 1990; Futreal *et al.* 1994; Miki *et al.* 1994) and the gene for cystic fibrosis (Riordan *et al.* 1989). As for model organisms, unbiased screens have led to surprises. For example, the search for genes involved in hypertension led to the unexpected finding that it results from changes in kidney function rather than heart function (Lifton 1995). As in studies of model organisms, multiple alleles of genes have been key in revealing the diversity of phenotypes that are possible for changes in a single gene. For example, screens for genes important in cancer led to the discovery of a series of p53 alleles that revealed the surprising functional complexity of p53 and helped to elucidate how its activities contribute to tumor suppression (Zilfou and Lowe 2009), potentially leading to allele-specific therapies (Yu *et al.* 2012).

Despite their value, unbiased mutant hunts have been limited to a relatively small number of model organisms among microbes, animals, and plants. Historically, mutant hunts have required the ability to look at large numbers of mutagenized individuals to find rare mutants, as well as the ability to map or complement mutations to go from mutant phenotype to genotype. However, this approach is less feasible in organisms with long generation times and large genomes, and impossible in others that cannot be mated. One popular approach to bypass mutant hunts in such organisms has been to inhibit expression of specific candidate genes by knockdowns or gene deletions. However, that is a limited approach that does not offer the virtue of unbiased mutant hunts, which by their nature explore broad swaths of biological space and lead to unanticipated new insights.

Technical revolutions over the last decade have reduced some of the most severe barriers to carrying out mutant hunts. Following in the footsteps of the *Saccharomyces cerevisiae* deletion set (Giaever *et al.* 2002), major progress for metazoans began with the development of genome-wide approaches for systematically knocking down gene function, initially with RNA interference (RNAi) libraries. Genome-wide RNAi screens, initially applied to *Caenorhabditis elegans* (Kamath *et al.* 2003) and then quickly adapted for *Drosophila*, plants, and mammalian cells (Cullen and Arndt 2005), enabled screening the majority of genes in large genomes, allowing rapid transition from phenotype to genotype. One example of countless successes of genome-wide RNAi screens has been the identification of genes involved in cancer (Kolfschoten *et al.* 2005; Westbrook *et al.* 2005). More recently, genome-wide, systematic screens of mutations generated by clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 have started to emerge (Shalem *et al.* 2014; Wang *et al.* 2014). The efficiency of CRISPR-mediated mutagenesis in creating homozygous mutations is a key aspect of mutagenesis of diploid cells (Shalem *et al.* 2015). These screens have identified genes involved in many aspects of human disease, for example: tumor growth (Chen *et al.* 2015), viral growth (Ma *et al.* 2015), and inflammatory diseases (Schmid-Burgk *et al.* 2016). The plethora of novel insights arising from these approaches is a clear demonstration of the potential of mutant hunts when a major hurdle to their execution is removed.

The current uses of CRISPR-mediated genome editing are only the tip of the iceberg of its potential to augment mutant hunts. First, as CRISPR technology is advancing, allele replacement in large genomes is quickly becoming routine, as it has been with yeast for decades. This will allow saturation mutagenesis of a specific gene (for example: Canver *et al.* 2015; Varshney *et al.* 2015) and it will enable screens for a complete allelic series. Allelic series provide unbiased means to assess the quality, complexity, and timing of a function by providing multiple classes of mutations, such as those that impair specific functions of multi-domain proteins. Importantly, the construction of different classes of conditional alleles will also become more feasible. Different types of conditional alleles have been critical reagents in the study of biological processes, such as the use of temperature-sensitive mutations to assess essential functions in cell division, or conditional cre-lox constructs to assess function during development or in disease. Furthermore, emerging evidence suggests that the ability to impose rapid loss of a function may be more revealing than a constitutive loss of the same function, as propagation of mutants with constitutive loss-of-function alleles often selects for compensating genetic (Rossi *et al.* 2015) or epigenetic (Wang *et al.* 2015) changes.

As CRISPR appears to work in most organisms in which it has been tried, we anticipate that the routine sequencing of genomes coupled with the use of genome-wide CRISPR screens will allow efficient mutant hunts in a multitude of complex organisms, well beyond those that are currently genetically tractable. We expect that over the next decade the number of model organisms will have increased dramatically. The expansion in the types of organisms and cells that are studied will mean that the phenotypic space we explore will no longer be constrained to the limited traits of current model systems (Bolker 2012) and will expand to areas such as cryptobiosis in tardigrades (Mobjerg *et al.* 2011), regeneration in *Planaria* (Reddien 2013), and important pathogens such as *Chlamydia* (Kokes *et al.* 2015).

While genome-wide RNAi and CRISPR screens greatly enhance the ability to find mutants, they restrict the possible spectrum of mutations that can answer a screen or selection to those that decrease or eliminate gene function. In contrast, spontaneous or chemically induced mutations allow one to survey a much greater fraction of mutational space. In the past, this virtue was offset for many organisms by the difficulty of identifying the causative mutation. This hurdle has been dramatically reduced by high-throughput sequencing and the ability to use CRISPR to test candidate genes for causality.

The ability to perform deep exploration of mutational space will be particularly valuable in allowing increased use of unbiased screens and selections for two classes of mutations that explore gene–gene interactions: suppressor and enhancer mutations. The isolation of suppressors has been one of the most powerful genetic approaches in model organisms; it will be a huge advance for organisms in which it is

currently infeasible or unavailable. The isolation of enhancer mutations, second mutations that enhance the phenotype of a mutation, has similarly been an important way to elucidate functional connections. Importantly, the isolation of enhancers can address the issue of functional redundancy, where nonhomologous proteins carry out overlapping functions.

The ability to characterize mutants will be augmented further by advancing technologies, such as the increasing ability to automate biochemical assays and microscopic imaging, enabling screens for mutant phenotypes previously thought to be too tedious or time consuming. In addition, advances in mass spectrometry will provide increasingly high-resolution mutant characterization by cellular and subcellular analysis of protein content, levels, and modifications, as well as levels of metabolites. Genome-wide approaches to analyze a mutant, such as RNA sequencing and chromatin immunoprecipitation with massively parallel DNA sequencing, will also increase in both the types of assays and their availability. With these greater capabilities, though, comes a large challenge: analysis and integration of huge data sets from many different types of assays. This need will clearly increase our reliance on computational skills and quantitative analysis. Figuring out which mutants to focus on and determining which phenotypes are the direct consequences of a mutation have been enduring challenges for geneticists; they will become even greater challenges as we learn more ways to identify and assay mutants. As geneticists, we will want to continue to aim our studies on the functions of individual genes and on how these functions relate to phenotype, as well as on leveraging genome-wide or cellular-wide data to elucidate networks of gene interactions.

One key area of genetics that will become considerably more approachable over the next decade is complex or polygenic traits. This is currently an area of great significance in model organisms, applied genetics, and human disease (Womack *et al.* 2012; Mackay 2014). Historically, complex traits have been studied among strains or individuals found in nature. The advances in the efficiency of mutagenesis and whole-genome sequencing after pooled linkage analysis (Brauer *et al.* 2006; Ehrenreich *et al.* 2010) will allow unbiased screens in the laboratory not only for single mutations that confer traits but for the *de novo* creation and analysis of polygenic mutants as well. This approach opens the door for the identification and analysis of phenotypes previously unapproachable in the lab (for example: Koschwanez *et al.* 2013).

In conclusion, because so much biology remains unknown in model organisms, in humans, and in the diverse species yet to be studied, many important and surprising discoveries await us. We believe that rewarding and productive mutant hunts, unleashed by new technologies that allow efficient generation of mutations and their identification by DNA sequencing, will be critical for these discoveries. The exciting future for genetics will be constrained only by our ability to recognize interesting new areas of investigation and by our creativity in designing and carrying out unbiased mutant hunts to address them.

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