SPEAKER SPOTLIGHT:

Using CRIPSR-Cas Technology in Leveraging *Drosophila* for Biomedical Research

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The *Drosophila* fly provides a relatively effective, quick and inexpensive model for human disease-related research. Using *Drosophila* in looking at CRISPR activation to 'up-regulate' genes is one way in which *Drosophila* RNAi Screening Center at Harvard Medical School is using these organisms in the gene editing area of biomedical research.

Q1: You have an unconventional role in academia. Can you tell us about what your group does?

Typical academic labs use a variety of techniques to study a given biologic topic. We flip this model. At the *Drosophila* RNAi Screening Center (DRSC), we are focused on development and optimization of new genetic technologies, and help other groups apply these technologies to study diverse topics in biology and biomedicine. The resources we develop, which include new transgenic fly stocks, cell lines, high-throughput screening libraries, protocols, and bioinformatics tools, are made broadly available to the research community and have been used in projects related to cell signaling, cell morphology, host-pathogen interactions, cancer biology, rare genetic diseases, innate immunity, neuroscience—the list goes on.

Q2: What genetic technology areas are you focused on now?

CRISPR-Cas technology development is a current focus area for us. Diving into CRISPR-Cas technologies was a natural extension of our existing platform, which had been centered around support for RNAi-based studies in flies and fly cells. We recently received NIH funding to form the *Drosophila* Research and Screening Center-Biomedical Technology Research Resource (DRSC-BTRR). As part of the DRSC-BTRR, we partner with laboratories that have specific technological development needs. These 'driving biomedical projects' focus our efforts and help ensure that the technologies we develop fill specific needs. We also have a *Drosophila* fly stock production platform that generates new fly resources based on established technologies, including production of thousands of single guide RNA (sgRNA) fly stocks useful for CRISPR knockout, and more recently, for CRISPR activation (CRISPRa).

Q3: Why CRISPRa?

Over-expression phenotypes can help reveal functions in a manner that is different from and complementary to what we learn from loss-of-function studies. For example, mis-expression of the *Drosophila eyeless* gene in non-eye tissues led to a striking result: eye-like patches of tissue, complete with red pigment, formed on legs, wings, or other places where expression of the gene was induced. These studies helped define the role of *eyeless* as a 'master control gene' able to turn on an entire differentiation pathway. Understanding *eyeless* turned out to have health relevance, as the human ortholog of *eyeless*, *PAX6*, is important for eye development in humans and like *eyeless*, interacts with an evolutionarily conserved network of other factors.

We cannot expect that over-expression of all or even most genes will lead to the kind of striking phenotypes and insights that were observed for *eyeless.* But results obtained using CRISPRa-based activation in *Drosophila,* including in specific tissues or stages, are likely to be informative. Over-expression of rate-limiting enzymes, for example, might help us dissect metabolic pathways and understand their broader impact on development and physiology.

Q4: How might CRISPRa specifically contribute to modeling human diseases?

It's a fair question. We hear a lot about loss-of-function disease models, for example to model recessive genetic disorders or loss of tumor suppressor genes in cancers. But not all genetic diseases are associated with loss or reduction of gene function. Our group has identified specific sets of genes for which gene activation might be an appropriate route to development of disease models. To do this, we start with human datasets. Transcriptomics analysis of cancers, for example, has helped identify human genes that are up-regulated in one or more cancer type. Experimental testing is needed to help prioritize and characterize these genes. We can test the hypothesis that up-regulation of a given gene promotes cell proliferation by using CRISPRa to overexpress an equivalent or 'orthologous' gene in *Drosophila*, then observing what happens. CRISPRa-based studies might also help identify what subset of genes associated with microduplication syndromes lead to symptoms. For that reason, we are also building sgRNA fly stocks designed for activation of fly orthologs of genes in duplicated regions. In either case, what's learned using CRISPRa in *Drosophila* might point to new mechanistic insights and identify new targets for therapeutics.

Q5: Why do you focus on *Drosophila?*

Drosophila is a surprisingly good model for human disease-related research. Flies have a brain, a gut, a beating heart, and tissues equivalent to kidneys, liver, fat, and more. They also exhibit an array of behaviors such as circadian rhythms, sleep, aggression, courtship, and predator avoidance that have parallels to our own behaviors, including at the level of genetic control. Although CRISPR-Cas technologies and techniques such as organoid formation allow for better and more complex models using human cells, barriers remain, including time and money. *Drosophila* studies are relatively inexpensive and fast. In addition, the lower level of gene redundancy in the *Drosophila* genome makes it possible to uncover functions that might be missed in equivalent studies in mammalian systems, and about 75% of human disease-associated genes have an equivalent in the fly.

The various 'flavors' of CRISPR-Cas systems and related technologies—which we and others are using for knockout, knock-in, activation, and other perturbations—are further improving the speed and accuracy with which we can develop new *Drosophila* disease models, perform genetic screens, test the impact of human variants, and uncover additional disease-relevant information. Add to this the many ways in which we can compare data from *Drosophila* with data from other systems, which are similarly benefitting from the CRISPR-Cas technology revolution, and I'm awed by the possibilities. We are in a better position than ever to leverage *Drosophila* as part of broader biomedical research efforts. It's an exciting time for the field.

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