

# Using *Drosophila melanogaster* To Discover Human Disease Genes: An Educational Primer for Use with “Amyotrophic Lateral Sclerosis Modifiers in *Drosophila* Reveal the Phospholipase D Pathway as a Potential Therapeutic Target”

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**ABSTRACT** Since the dawn of the 20th century, the fruit fly *Drosophila melanogaster* has been used as a model organism to understand the nature of genes and how they control development, behavior, and physiology. One of the most powerful experimental approaches employed in *Drosophila* is the forward genetic screen. In the 21st century, genome-wide screens have become popular tools for identifying evolutionarily conserved genes involved in complex human diseases. In the accompanying article “Amyotrophic Lateral Sclerosis Modifiers in *Drosophila* Reveal the Phospholipase D Pathway as a Potential Therapeutic Target,” Kankel and colleagues describe a forward genetic modifier screen to discover factors that contribute to the severe neurodegenerative disease amyotrophic lateral sclerosis (ALS). This primer briefly traces the history of genetic screens in *Drosophila* and introduces students to ALS. We then provide a set of guided reading questions to help students work through the data presented in the research article. Finally, several ideas for literature-based research projects are offered as opportunities for students to expand their appreciation of the potential scope of genetic screens. The primer is intended to help students and instructors thoroughly examine a current study that uses forward genetics in *Drosophila* to identify human disease genes.

**KEYWORDS** neurodegeneration; ALS; *Drosophila*; genetic screen; education

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ONE of the most powerful approaches for discovering the functions of genes is the forward genetic screen. Screens in model organisms like *Drosophila melanogaster* can identify evolutionarily conserved genes involved in complex human diseases. As an example, in the accompanying article “Amyotrophic Lateral Sclerosis Modifiers in *Drosophila* Reveal the Phospholipase D Pathway as a Potential Therapeutic Target,” Kankel and colleagues describe a forward genetic screen to discover factors that contribute to the severe neurodegenerative disease amyotrophic lateral sclerosis (ALS). This primer guides students through the data in the article and offers ideas for literature-based research projects to expand students’ appreciation of genetic screens.

### Model Organisms

It is impossible to imagine the successes of modern medicine without the contributions from so-called “model organisms.” Because fundamental genetic and cell biological processes are common to all life on Earth, profound insight into human biology can be gained by studying species simpler than our own. Research on organisms as distant from us as bacteria and their phages have helped provide the thorough and extensive understanding we have today of the genetic material, the code by which it stores information, and the process by which this information is decoded by cells. That foundational knowledge, built in model organisms, laid the groundwork for the molecular biology revolution of the mid-20th century, which in turn underpins the modern biomedical enterprise. Likewise, animal studies in rats, mice, chicks, frogs, fish, and invertebrates have been providing insight into the evolutionarily conserved mechanisms of our own development, physiology, and behavior, and related pathologies, for well over a century. In their recent article, Kankel and coauthors report on a genetic study using the fruit fly, *D. melanogaster*, as a model organism (Kankel *et al.* 2020).

### *Drosophila melanogaster*

*D. melanogaster* has a particularly long history as a model organism, having first achieved fame in the early 20th

century for its role in establishing the chromosome theory of inheritance. Because of its long history, its resurgence in popularity starting in the mid-20th century, and its “user-friendly” attributes, including short life-cycle, small size, high fecundity, and relatively compact genome, *Drosophila* has become a research platform with a plethora of experimental tools (Hales *et al.* 2015). These tools, coupled with the fact that more than half of *Drosophila* genes have orthologs in humans, have made the fruit fly a leading model system for studying the mechanisms of human biology and disease (Bellen and Yamamoto 2015). Additionally, its complex brain and behavioral repertoire make it a particularly useful model for understanding neurobiology (Bellen *et al.* 2010; McGurk *et al.* 2015).

### Genetic Screens

After early experiments in *Drosophila* helped elucidate the rules of inheritance and the nature of genes, studies in this organism turned to the question of how genes direct biology. By examining what happens when genes are mutated, scientists have been able to tease apart how unmutated wild-type alleles contribute to the normal functioning of an organism. Since the 1960s, forward genetic screening has been used to identify novel genes involved in myriad biological processes (St Johnston 2013). Forward genetic screens begin by creating *de novo* mutations randomly throughout the genome, so that any gene has a chance of being altered (“hit”) and the researcher is unbiased toward any specific gene or class of genes (St Johnston 2013; Hales *et al.* 2015). Mutants then are surveyed for distinct phenotypic alterations as compared to the wild type. When applied on a large scale, this powerful method has the potential to capture any, and theoretically all, genes involved in a particular process (Nüsslein-Volhard and Wieschaus 1980; St Johnston 2002). In contrast to “reverse” genetics approaches, in which a specific gene or group of genes is targeted *a priori*, forward genetic screens are uniquely poised to discover pathways previously unknown to be involved in the process of interest. Some of the earliest screens centered on stereotyped behaviors, thereby demonstrating that even biological functions as complex as

neurobiology and behavior are regulated by the activity of specific genes (e.g., Benzer 1967; Hotta and Benzer 1969; Pak *et al.* 1969; Jan and Jan 2008).

### Dominant Modifiers

One of the most obvious physical features of the *D. melanogaster* fly is its eye—a large, complex, and beautiful neurological organ. Since the very first described *Drosophila* mutant with white eye color instead of red, some of the most notable forward genetic screens have looked for mutations that perturb the eye (Morgan 1910; St Johnston 2002). A number of these screens take advantage of the fact that genes in the same pathway have a tendency to be sensitive to each other's dosage (e.g., Rogge *et al.* 1991; Simon *et al.* 1991). Because changes to the sequence of nucleotide bases in a gene often reduce the functionality of the encoded gene product (protein or RNA), many mutant alleles are loss of function. In diploid organisms, including *Drosophila* and humans, a single wild-type allele typically produces enough gene product to maintain normal activity; hence, most loss of function mutant alleles are recessive and must be homozygous to affect phenotype. However, if another component of the same pathway is altered in its genetic dosage, loss of function mutant alleles can exacerbate (enhance) or ameliorate (suppress) the phenotype as heterozygotes, and thus appear dominant. In this way, forward genetic modifier screens can be performed by changing the dosage of one pathway component, thereby creating a sensitized background, generating novel mutations randomly throughout the genome, and then screening for those second-site alleles that are dominant modifiers of the starting phenotype. This enables identification of genes that act in the same pathway as the original component, with the added advantage that the screen can be performed with only one generation of flies, as the dominant modifiers do not need to be homozygous to manifest the altered phenotype. This strategy is especially valuable because mutant alleles that are otherwise homozygous lethal can be identified as heterozygotes, with survival of the organism permitted. Furthermore, the eye, being a non-essential organ, is an ideal context in which to study development and physiology without impacting survival or fertility (Baker *et al.* 2014). In their study, Kankel *et al.* (2020) perform a genetic screen for dominant modifiers of a mutant eye phenotype.

### Expressing Human Genes in *Drosophila*

The experimental range of *Drosophila* was expanded vastly by transgenic technology. Once the tools of molecular biology made it possible to isolate individual genes from the genome and engineer them in bacterial plasmids, methods were developed to deliver any gene of interest into the genome of another organism, of the same or different species (Rubin and Spradling 1982; Spradling and Rubin 1982). With this technology in hand, human genes now could be introduced into *Drosophila* (e.g., Jowett *et al.* 1991; Luo *et al.* 1992). In the

decades since, this procedure has been used to demonstrate time and again the strong evolutionary conservation between flies and humans, with human proteins capable of rescuing mutant phenotypes conferred by loss of their fly counterparts. Furthermore, expressing human disease alleles in flies often mimics disease pathology, again highlighting the robust underlying conservation of genetic pathways and cellular networks (e.g., Jackson *et al.* 1998; Warrick *et al.* 1998). Kankel *et al.* (2020) use this approach of expressing human disease alleles in the fly eye, which creates a visible mutant phenotype.

### GAL4-UAS

In order for an exogenous transgene to be expressed in the host organism, it must include regulatory sequences for transcription and translation. Early transgenes were engineered with specific *Drosophila* regulatory elements, but the GAL4-UAS system introduced a great technological improvement by separating the gene of interest from its regulation (Brand and Perrimon 1993). In this system, *Drosophila* transgenes are engineered downstream of an upstream activation sequence (UAS) from the *Saccharomyces cerevisiae* genome that binds the GAL4 transcription factor. Because the *Drosophila* genome does not endogenously encode GAL4, UAS-driven transgenes are effectively not expressed, thereby protecting the transgenic organisms from expression of deleterious transgenes. A vast array of separate transgenic *Drosophila* stocks has been generated and shared within the scientific community, each carrying the yeast GAL4 transcription factor expressed in a tissue- or cell-specific pattern under the control of an endogenous regulatory element (Hales *et al.* 2015). Thus, any UAS-driven transgene now can be expressed in almost any tissue or cell type by simply mating to a GAL4 stock. Kankel *et al.* (2020) use the *GMR-GAL4* driver, which produces GAL4 protein in cells of the eye, to induce expression of UAS-controlled human disease alleles.

### Genome-Wide Mutant Collections

By the last decade of the 20th century, the fly research community was inspired to generate new collections of mutants that could be used for rapid forward genetic screening and subsequent mapping (Cooley *et al.* 1988; Spradling *et al.* 1995). Collections aiming to represent every gene in the genome made use of mobile transposable elements that could integrate throughout the genome at random, creating loss of function mutations by disrupting local regulatory and/or coding sequences (Artavanis-Tsakonas 2004; Thibault *et al.* 2004; Bellen *et al.* 2011). In this study, Kankel *et al.* (2020) screen one of these collections of *Drosophila* insertion mutations, the Exelixis collection. These insertions can be mapped immediately by isolation of the inserted element along with its neighboring genomic DNA, greatly reducing labor- and time-intensive procedures for mapping hits from screens. The utility of collections like these has been augmented further by whole genome sequencing, which revolutionized the

study of biology at the turn of the 21st century (Adams *et al.* 2000). Today, scientists have at their fingertips nearly the entire *Drosophila* genome sequence, along with libraries of mutants disrupting a substantial proportion of protein-coding loci, making possible rapid genetic screening, identification, and functional analysis. The availability of the human genome sequence, together with the revelation that 60%–70% of human genes have orthologs in *Drosophila*, and that this percentage is even higher (~75%) for disease genes, has prompted the scientific community to use the powerful tools of *Drosophila* genetics to understand human disease (Lander *et al.* 2001; Venter *et al.* 2001; McGurk *et al.* 2015; Wangler *et al.* 2017; Johnston 2020).

## Organizing Screen Hits

A successful large-scale genome-wide genetic screen can identify hundreds of hits, genes whose mutation leads to interesting phenotypic alterations. To organize these hits, scientists often use publicly available gene ontology (GO) classification, which annotates each gene with molecular functions, biological processes, and cellular sites of activity (<http://geneontology.org/>). Annotations are based on data from many different model systems, as well as on computer-based analyses, and are curated by a large global consortium of scientists (Gaudet *et al.* 2017). This diversity of inputs, along with a controlled vocabulary that is clearly defined and adheres to a consistent logical framework, makes the GO classification structure universally applicable (Hastings 2017). GO classification can help group screen hits according to function and/or cellular localization, thereby illuminating biological pathways important for the process under investigation.

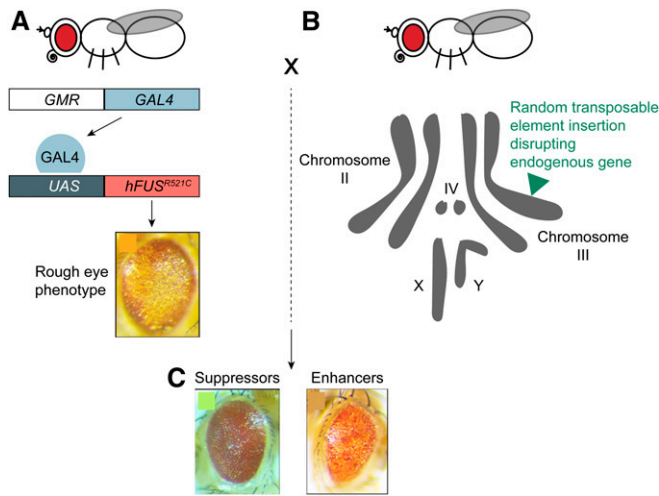
## Amyotrophic Lateral Sclerosis

Human life expectancy has increased dramatically over the course of the last century due largely to improved nutrition, public health, and medical care, but with increased lifespan comes greater risk of age-associated diseases, including neurodegenerative disease (ND, <https://www.nia.nih.gov/research/dbsr/global-aging>). ND encompasses a range of conditions that cause the death of neurons and loss of neurological function, including Alzheimer's disease, Lewy body diseases like Parkinson's, polyglutamine diseases like Huntington's disease, and amyotrophic lateral sclerosis (ALS). Despite much investigation, a comprehensive understanding of ND has been confounded by the complex contribution of a variety of genetic and environmental factors. ALS, commonly known as Lou Gehrig's disease, is a severe ND in which motor neurons die and their target muscles atrophy (Brown and Al-Chalabi 2017). Although a modest proportion of ALS cases are clearly inherited (familial ALS, fALS), ~80%–90% of ALS cases are sporadic (sALS), with only ~20% of sporadic cases linked to specific genes (Vucic *et al.* 2014; Brown and Al-Chalabi 2017; Martin *et al.* 2017). This, together with the limited treatment options, creates a pressing need to understand better the genetic and

cellular pathways underlying this serious disease. Because fALS and sALS bear high clinical and pathological similarity, and because the identified genetic loci are common to both, it is presumed that fALS and sALS share molecular and cellular etiologies (Brown and Al-Chalabi 2017; Martin *et al.* 2017). Numerous genes have been identified in fALS (<https://alsod.ac.uk/>), with four loci accounting for the majority of cases, namely *FUS*, *TARDBP/TDP-43*, *C9orf72*, and *SOD1* (Brown and Al-Chalabi 2017; Hardiman *et al.* 2017). Interestingly, *FUS* and *TDP-43* are both RNA-binding proteins, and abnormal RNA processing may contribute to disease progression (Ranganathan *et al.* 2020; Yerbury *et al.* 2020). Still, ALS-associated mutations in *FUS* or *TDP-43* increase the tendency of the encoded proteins to misfold and aggregate abnormally, a property likewise observed for ALS-associated *SOD1* mutants, suggesting that dysregulation of protein homeostasis (proteostasis) and resultant proteotoxicity is the cause of neurodegeneration (Martin *et al.* 2017; Yerbury *et al.* 2020). In support of this idea, *TDP-43* aggregates are found in up to 97% of ALS patients, representing both sporadic and familial cases, including in patients with wild-type *TDP-43* alleles (Hardiman *et al.* 2017). Toxic aggregates are also a feature of ALS linked to mutations in *c9orf72*. The protein encoded by *c9orf72* is implicated in autophagy, an important cellular process for regulating proteostasis, but ALS-causing mutations derive from expansion of a hexanucleotide GGGGCC repeat in a noncoding intron of the gene. These repeated sequences are transcribed into RNAs that aggregate and sequester RNA binding proteins, and the repeat containing RNAs can be translated via a noncanonical protein translation pathway into dipeptide repeats that form toxic protein aggregates (Ranganathan *et al.* 2020). Much is still unknown about how ALS develops, including whether and how dysregulation of RNA processing and proteostasis lead to neurodegeneration, whether ALS-associated mutations cause disease by loss of their wild-type functions or gain of toxicity, and whether these four genes, as well as others, act in the same genetic and biochemical pathway. Moreover, despite the prevalence of RNA processing defects and proteotoxic aggregates in ALS, many other cellular activities are affected as well (Hardiman *et al.* 2017). The goal of this study is to link previously unassociated genes to ALS, in order to better understand how the disease progresses and to reveal potential therapeutic targets. Prior studies have demonstrated that expressing human disease variants of *FUS* or *TDP-43* with the GAL4-UAS system in photoreceptor neurons of the *Drosophila* eye induces degeneration, mimicking the cellular pathology of the disease (Ritson *et al.* 2010; Larson *et al.* 2011). The authors screen the Exelixis collection of *Drosophila* insertion mutations for dominant modifiers of this eye degeneration phenotype, to elucidate the molecular pathways leading from the human disease alleles to ALS pathology (Figure 1).

## Unpacking the Work

In order to conduct genome-wide screens for ALS genes, the authors develop a strategy to cross transgenic *Drosophila*



**Figure 1** To conduct their screen, the authors collected flies from (A) a *Drosophila* strain carrying an ALS-associated allele, for example *hFUS<sup>R521C</sup>*, under control of a UAS regulatory element along with the *GMR-GAL4* driver. Expression of *UAS-hFUS<sup>R521C</sup>* with *GMR-GAL4* causes a degenerative rough eye phenotype. These flies were crossed to (B) flies from the Exelixis collection, each carrying an individual mutation caused by random insertion of a transposable element in the genome (green triangle). F1 progeny (C) were examined for enhancement or suppression of the rough eye phenotype.

strains expressing ALS-associated human alleles to insertion mutations from the Exelixis collection and to examine the F1 progeny (Figure 1). Females were collected from *Drosophila* strains carrying either of two ALS-associated alleles, *hFUS<sup>R521C</sup>* or *hTDP-43<sup>M337V</sup>*, each controlled by a UAS regulatory element. Each strain also carries the *GMR-GAL4* driver, which produces GAL4 protein in cells of the eye, thereby causing expression of the human ALS-associated proteins there. These females were mated to males from the Exelixis collection, each strain of which carries an individual insertion mutation.

All starting strains also carry balancer chromosomes [see (Hales *et al.* 2015) for a detailed description of balancer chromosomes], which allow stocks to be maintained as heterozygotes by bearing recessive lethal mutations and suppressing meiotic recombination with homologous chromosomes. The balancers are marked with the dominant wing phenotype Curly (Cy, curly wings instead of straight, on the balancer CyO), or the dominant larval phenotype Tubby (Tb, larvae are shorter and fatter than wild type, on the balancer TM6B). The second chromosome balancers in the strains expressing the human ALS alleles additionally carry the yeast gene *GAL80* expressed in all cells. *GAL80* protein inhibits *GAL4* activity, preventing expression of the ALS alleles in the starting strains (St Johnston 2013).

## Technical Glossary

This glossary provides some technical information to assist with understanding the experiments in the research article.

**RNAi (RNA interference)** uses a small double-stranded RNA molecule to target an endogenous mRNA for

post-transcriptional silencing. To employ RNAi *in vivo*, a transgene is generated encoding an RNAi transcript under control of a UAS regulatory element, allowing directed expression by the GAL4-UAS system. The transgene is designed with sequence complementarity to an endogenous target mRNA and an inverted repeat structure, which facilitates folding of the RNAi transcript into a double-stranded RNA. The double-stranded RNA promotes destruction of the target mRNA with matching sequence, thereby inhibiting production of functional protein from the target gene. This process of post-transcriptional knockdown often produces similar phenotypes as loss of function alleles and circumvents the time and labor needed to create genomic mutations. Furthermore, the *Drosophila* research community has produced libraries of RNAi transgenes targeting every gene in the genome, which are readily available (Mohr and Perrimon 2012).

**Imaginal discs** are epithelial sacs of primordial cells that give rise to the external anatomical structures of the adult fly, also called the imago (Beira and Paro 2016). For example, the eye imaginal discs are the precursors of the adult eyes. Imaginal discs are specified during embryogenesis, are patterned and grow during larval development, and form their distinct morphologies during metamorphosis.

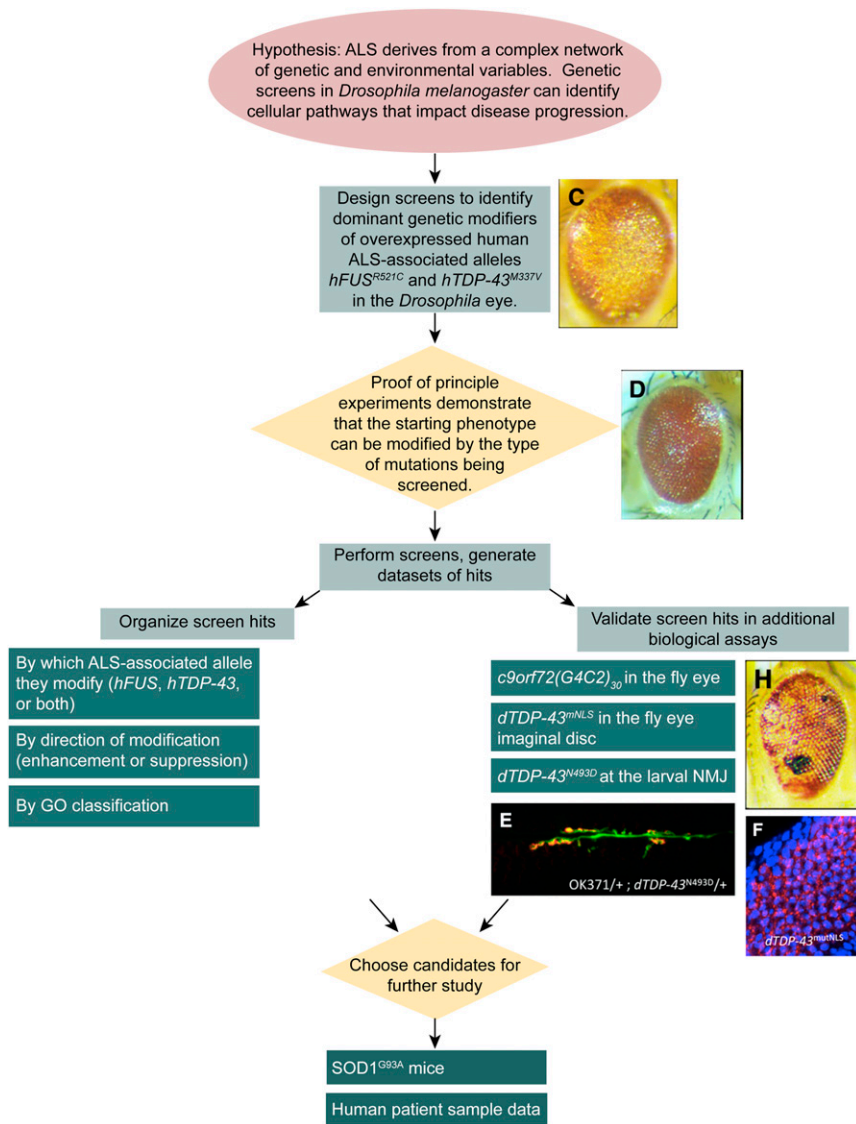
**The neuromuscular junction (NMJ)** is the site of contact between motor neurons and muscle tissue. In the *Drosophila* larva, motor neuron axon terminals at the NMJ form synaptic boutons, round structures that contain the synaptic active zones. Each *Drosophila* motor neuron creates a stereotypical number of boutons, providing a quantitative measurement of the fidelity of NMJ development and maintenance (Menon *et al.* 2013). In ALS, motor neuron degeneration is accompanied by disassembly of the NMJ and atrophy of muscle tissue (Cappello and Francolini 2017).

**A dominant negative allele** is one that interferes with the activity of the wild-type allele. Dominant negative alleles are functionally dominant, *i.e.*, they generate a mutant phenotype as heterozygotes, but because their mutant phenotypes result from reduced activity of the wild-type allele, they are loss of function.

## Guided Reading Questions

The guided reading questions below are intended to help students work through the results of Kankel *et al.* (2020). Students can be expected to spend ~15–30 min on each figure, on average. Questions can be assigned as homework in preparation for class discussions or as in-class group work. The overall logic of the experiments presented in the article is diagrammed in Figure 2. Answers to the guided reading questions are available as Supplemental Material online.





**Figure 1.** A genome-wide screening strategy for dominant modifiers of human ALS-associated allele induced eye degeneration in *Drosophila*.

1. What is the purpose of the screens described in this article? How were the screens conducted?
2. Explain each of the starting *Drosophila* strains used for the screens, shown in Figure 1A of Kankel *et al.* (2020). What genetic elements does each starting strain carry?
3. Draw Punnett squares representing the screen crosses. Which are the desired progeny and how will they be selected?
4. Which photomicrograph panel shows a normal fly eye? How do Figure 1, C and H compare to normal?
5. Describe what is shown in Figure 1, D–G and I–L in your own words. For each of the four genes shown, *dSETX*, *dco*, *Hsc70Cb*, and *Ask*, explain how it was tested and whether it is a suppressor or enhancer of *hFUS*<sup>R521C</sup> and *hTDP-43*<sup>M337V</sup>.

**Figure 2.** Workflow diagram illustrating the logic of the screening process. Genetic screens begin with a hypothesis, *i.e.*, that genetic screens in *Drosophila* can be used to identify novel genes associated with ALS. Because human ALS-associated alleles *hFUS*<sup>R521C</sup> or *hTDP-43*<sup>M337V</sup> cause a visible degenerative eye phenotype in adult *Drosophila*, libraries of mutants like the Exelixis collection can be screened for dominant modifiers of this phenotype. Once the screens have been planned, proof-of-principle experiments are conducted to demonstrate that the screen has the potential to be successful, using selected *Drosophila* mutants of other ALS gene orthologs as positive controls. The screens are then performed, and a list of hits is generated. The list can be organized by which ALS allele is affected by each modifier, whether modifiers are enhancers or suppressors, and according to GO classification for biological function and/or cellular localization. Screen hits also are validated experimentally by assaying whether they modify other ALS model systems, including *c9orf72(G4C2)*<sub>30</sub>-mediated degeneration of the adult *Drosophila* eye, *dTDP-43*<sup>mNLS</sup> aggregation in the larval eye imaginal disc, and *dTDP-43*<sup>N493D</sup> perturbation of the larval NMJ. Candidates that show activity in the secondary assays may be chosen for further study, which includes examination in an ALS model mouse and in human ALS patient data.

**Figure 2.** Dominant modifiers of human ALS-associated allele induced eye degeneration are identified in the screens.

1. How many insertion mutations were screened? How many crosses were established in the screens?
2. How old were the F1 flies when they were screened?
3. How many hits were recovered from the screens? What percentage of the total do the hits represent? Show your calculations. Is this percentage higher, lower, or on par with expectations?
4. How many hits affect both *hFUS*<sup>R521C</sup> and *hTDP-43*<sup>M337V</sup> transgenes? Do those common hits always affect the two transgenes in the same way? Give an example and propose a hypothesis to explain these results.

**Figure 3.** Validating screen hits using another ALS genetic model.

1. Explain the experiment shown in Figure 3. How does it differ from the original screens? What are the advantages

- and disadvantages of this experiment compared to the original screens?
2. What does the graph in Figure 3I show? How is each percentage calculated?
  3. What percentage of genes tested shows effects in this experiment?

**Figure 4. Validating screen hits using another ALS-associated phenotype.**

1. How do the transgenes in the experiment shown in Figure 4 differ from those in the experiments shown in Figure 1?
2. Compare and contrast the proteins expressed from these transgenes with the original constructs. What additional disease-relevant property do these proteins show?
3. Is this additional property modified by the hits from the screens? What does that suggest about how this property relates to degeneration?

**Figure 5. Validating screen hits in another cellular context.**

1. In Figure 5, the authors switch their focus away from the eye. What anatomical structure is the focus of this figure? In which cells is *OK371-GAL4* expressed? Why do the authors choose to examine this structure?
2. The authors use the *OK371-GAL4* driver to express three different *dTDP-43* variants. Among these three variants, which one has the most severe mutant phenotype and how do you know? How does this relate to the anatomical structure shown in the photomicrographs? What does this suggest about this variant?
3. The authors test three genes, *SF2*, *lilli*, and *kfp98A*, for their ability to modify the phenotypes caused by *OK371-GAL4* expression of *dTDP-43*. How were these three genes chosen?
4. Which of these three genes has a significant effect on the *OK371-dTDP-43* phenotype? Does any of the three fail to affect the *OK371-dTDP-43* phenotype? Propose a hypothesis to explain these results.

**Figure 6. Phospholipase D is an important player in ALS.**

1. From their screens, the authors discover the Phospholipase D (PLD) pathway (schematized in Figure 9). How do they test the importance of PLD in ALS in Figure 6? Describe five results shown in this figure that corroborate the importance of PLD.
2. What effect would you expect if *Drosophila* RalA expression were reduced instead of PLD in the same type of experiments?

**Figure 7. The Phospholipase D pathway is important for ALS progression.**

1. Is your expectation above (Figure 6, question 2) confirmed? Explain why or why not.
2. How do the results in Figure 7 further bolster the importance of the PLD pathway in ALS?

3. Based on the experiments presented in Figures 6 and 7, are PLD pathway effectors likely to function upstream or downstream of *FUS*, *TDP-43*, and *c9orf72* in ALS disease development?

**Figure 8. Validating Phospholipase D in another model organism.**

1. What are the advantages of performing genetic screens in *Drosophila melanogaster* as opposed to mice? What are the advantages of testing genetic interactions in mice as opposed to *Drosophila*?
2. Compare the grip strength of wild-type mice, *SOD1<sup>G93A</sup>*-expressing mice, and *SOD1<sup>G93A</sup>*-expressing mice with mutations in *PLD1*, *PLD2*, or *PLD1* and 2. How does mutating *PLD* modify the effects of *SOD1<sup>G93A</sup>* expression?
3. Are the results shown in Figure 8 consistent with those shown in Figure 6? Why or why not?

## Putting It All Together

1. Other recent research articles have found that PLD pathway genes are upregulated in sALS patients with early-onset disease (Rabin *et al.* 2010; Kaplan *et al.* 2014). Is this finding consistent with the data presented in Figures 6–8? Explain.
2. Do you think the PLD pathway is a worthwhile therapeutic target for ALS? Why or why not?
3. Propose an experiment to follow up on the idea of PLD as a therapeutic target for ALS.

## Student Projects

After reading the article and working through the guided reading questions, instructors may choose to give their students the opportunity to develop a project of their own on a related topic. Below are two different options for student-directed projects. We recommend giving students several weeks to complete the assignment and submit in midsemester or at the end of the term. Projects can be formatted as a written paper or as an oral presentation, with students working as individuals or in groups.

1. Choose a disease besides ALS that interests you. Does this disease have a genetic component? Has this disease been modeled in *Drosophila* or another model organism? Design a genetic screen to identify novel factors in this disease.
2. Using PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), find another research article that describes a screen for ALS components. Compare and contrast with this article, using the questions below to help you.
  - a. What model organism or model system was used in your article?
  - b. How was ALS simulated in your article? What phenotypic aspects of ALS were examined?

- c. What are the advantages and disadvantages of the model system in your article compared to the one used in Kankel *et al.* (2020)?
- d. How was the screen conducted?
- e. What were the outcomes of the screen?
- f. Are there any screen hits in common between your article and Kankel *et al.* (2020)? Why might this be? What does this suggest?

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