F-box genes in *Drosophila* melanogaster: A Review

Shreya Vijay

Abstract:

The archipelago gene (*Ago*) in *Drosophila* melanogaster prevents cell division. The gene Fbw7 (the mammalian equivalent of *Ago*), suppresses tumorigenesis, which is the process that can indicate whether cancer is present. *Ago* is essential in shaping the embryonic tracheal system in *Drosophila* post-mitosis. As current research reflects, *Ago* encodes for an SCF-type polyubiquitin ligase which inhibits tumor growth. However, research has found that it is probable that *Ago* has other protein targets. Researchers have discovered the Trachealess (Trh) protein, which *Ago* targets. However, there are mutations of *Ago* that elevate Trh levels in-vivo, making them ineffective in binding Trh in a Dysfusion positive cell. (Dysfusion is a gene found in *Drosophila* which controls the tracheal fusion event). It has been noted that *Ago* plays a role in tracheal morphogenesis, which is the process by which tracheal cells attach to one another. Despite their attachment in tracheal morphogenesis, these cells will continue to remain flexible, which will allow for rearrangement during different phases of development in *Drosophila*. *Ago* has noteworthy functions in cells that have undergone mitosis; such data can be utilized to highlight *Ago*'s role in disease and cell development. This review article intends to analyze the function of *Ago* in Dysfusion-positive cells. It will study various experiments on *Drosophila* that have allowed researchers to uncover the relationship between the Trh protein, *Ago,* and the Dysfusion protein.

Introduction:

Why is Drosophila an effective model organism?

In 1909, researcher Thomas Hunt Morgan began experimenting with *Drosophila melanogaster* as a model organism in his experimental studies of evolution. His experiments were intended to oppose Mendelian genetics, and he induced mutations in *Drosophila* by altering selective pressures. Morgan discovered a white-eyed *Drosophila* in his collection of red-eyed flies. After discovering this, Morgan dropped his evolutionary experiments and started to analyze Mendelian genetics once more. (Tolwinski 2017). *Drosophila* proved valuable in these experiments due to its short life span and similarities in DNA to humans, allowing it to be an effective model organism. His discoveries led to the synopsis that some genes are not inherited independently and must be linked– in the case of the white eye, the trait was linked to the X chromosome.

Later, the *Drosophila* embryo was analyzed and led to a number of different breakthroughs that once again redefined genetics. These studies led to the conclusion that discrete genes led to different aspects of development (Papagiannouli, Mechler 2013). To clarify, discrete genes are genes that are controlled by a very small number of genes. These developments led to the belief that any human gene or allele could be studied in *Drosophila.*

With its distinct similarity in DNA between humans and *Drosophila*, sharing over 60% of genetic makeup, *Drosophila* has become an effective model system (3). This is in part due to its short life cycle and easy maintenance, allowing research labs to study complicated human processes quickly and easily (Tolwinski 2017). For nearly 100 years, researchers have been using *Drosophila* as a model system in their labs. Yet, there is still so much that this model

organism can tell us about the human body, and one such example of a new development in the study of *Drosophila* is its usefulness in studying tumorigenesis (Medina, Calleja, Morata 2021).

What is tumorigenesis?

Tumorigenesis is the process by which cancer is formed. This is caused by uncontrolled cell growth due to a malfunction in the cell cycle checkpoints which allows cancerous cells to grow quickly.

Drosophila's usage in studying cancer has come a long way (Herranz, Cohen 2017). Some of the first tumor suppressor genes were found in *Drosophila.* In 1967, scientist Ed Lewis identified the *lgl* mutant gene in wildtype *Drosophila.* Since then, various tumor suppressor genes have been discovered in *Drosophila*, further aiding in scientists' study of tumorigenesis. One such gene, the *Ago* gene, will be the subject of this review's discussion.

In depth perspective into current study of Ago and Trh

The *Ago* gene is a tumor suppressor gene which encodes for the F box component of an SCF ubiquitin ligase. Archipelago regulates Cyclin E and Notch activity, degrading pathways that control cell growth and the cell cycle (Interactive Fly: Gene Brief). The gene also encodes for a protein that inhibits tissue growth. To summarize past studies, *Ago* is heavily involved in cell-cycle regulation. Its mammalian equivalent, *Fbw7*, acts the same way.

Fbw7 is a human tumor-suppressor gene, and it is well established in regulating the cell cycle. Loss of *Fbw7* function leads to tumorigenesis, which can develop into cancer (Welcker, Clurman 2008).

The Trachealess, *Trh*, protein regulates tracheal morphogenesis in *Drosophila*. Tracheal morphogenesis is the process by which *Drosophila* makes new tracheal cells. This gene encodes a bHLH-PAS transcription, and is also one of the first genes that is expressed when new tracheal cells are formed (Chung, Chavez, Andrew 2011) This protein also has a mammalian equivalent, NPAS-3, which encodes for lung development. While scientists originally believed that the Trachealess protein played an insignificant role in *Drosophila*, recent studies have found that this protein is essential for the expression of every single tracheal gene. Relating *Trh* and *Ago* together, researchers have found that flies that do not carry the *Ago* gene have higher dorsal trunk break percentages, resulting in the theory that there is a connection between *Ago* and *Trh*, which this article will later touch on in the methodology, results and discussion portions of the paper.

To clarify one more point, it is essential to understand the process of Dysfusion in *Drosophila*. Dysfusion is a gene that allows for tracheal fusion in *Drosophila*. There is an inverse relationship between the Dysfusion and *Trh* proteins in wild type cells. As the level of Dysfusion rises, the levels of *Trh* decline (Jiang, Crews 2003).

The "argument."

This review intends to highlight the evolution in scientific studies of the *Ago* gene and how it affects various systems in *Drosophila.*

Methodology:

2007: Nathan T. Mortimer and Kenneth H. Moberg explore how the Archipelago gene controls the Trachealess transcription factor in the embryonic tracheal system.

The intention behind Nathan T. Mortimer and Kenneth H. Moberg's experiment was to identify the relationship between the *Ago* and Trh genes. The results will be explained later on in the review. Right now, we will focus on the methodology behind their study.

In their experiment, they used *ago1/ago3* trans-heterozygotes. Among other alleles, they also cloned *ago delta F* (a version that does not have a core F box domain) and formed UAS-*ago* and UAS-*ago delta F* stocks (Mortimer, Moberg 2007). They made statistical comparisons using Student's t test– which is used to compare means between two groups. This test will determine whether a difference in response between two groups is statistically significant or not (Mishra, Singh, Pandey 2019).

Embryos were staged and the samples were then rehydrated and washed in PBS. Later, they were incubated with mouse anti-Tango, mouse mAb2A12, rabbit anti-β-Gal, guinea pig anti-full length Ago, rat anti-Dys and rabbit anti-Dys primary antibodies. The extracts were prepared in buffer solutions with DTT and resolved in 7.5% SDS-PAGE prior to Western blots that were performed with rat anti-Trh or anti-β-tubulin (Mortimer, Moberg 2007).

They also performed RNA analyses. Researchers placed embryos in 1.5% formaldehyde-saturated heptane, devitellinized, and stored in methanol at -20 degrees Celsius. The embryos were washed in PBS, treated with Proteinase K for 2 minutes and then post-fixed with 4% formaldehyde. Further immunohistochemistry was performed as needed (Mortimer, Moberg 2007).

2009: Nathan T. Mortimer and Kenneth H. Moberg discovered how embryonic tracheogenesis is regulated in Drosophila.

In their experiment, these scientists revealed that there is an early stage of tracheal development that is vulnerable to hypoxia, which is an important breakthrough to understand how *Drosophila* responds to hypoxia– that is, when *Drosophila* is in an oxygen-deprived environment.

Their methods included cloning the *dVHL* open reading frame into a PCR product in the EcoRi site of the *pSymp* vector, which generated *UAS-dVHL* stocks. They genotyped embryos with 'blue' balancers and the *CyO, P {ActGFP}JMR1* balancer. Hypoxia treatments were performed using 0.5% O2: 99.5% N2 gas in a hypoxic chamber. O2 concentration was monitored with an electrical oxygen sensor (Mortimer, Moberg 2009).

Similar to their last experiment, they also used RNA in situ hybridization. Their results will further be analyzed in the "Results" section of this article.

2012: Wen Dui, Wei Lu, Jun Ma and Renjie Jiao phenotypically screen F-box genes through a RNAi-based approach.

In their experiment, Dui, Lu, Ma and Jiao created a dataset, analyzing phenotypes in the eye, wing and notum of *Drosophila.* Their final product created a datasource that provides future researchers with information about the molecular and genetic functions of *F-box* genes in *Drosophila.*

To establish this relationship, the scientist gathered fly stocks from the Vienna *Drosophila* RNAi center, the Fly Stocks of National Institute of Genetics and the Bloomington *Drosophila* Stock center. The flies were fed cornmeal, soybear, yeast agar, syrup and molasses and were cultivated between 22-25 degrees Celsius (Dui, Lu, Ma, Jiao 2012).

To study the function of the *F-box* genes, the scientists did a survey of literature and databases to list a total of 45 *F-box* genes.This allowed them to analyze phenotypes and establish the function of *F-box* genes, such as *Ago*. Their results will be discussed further in the "Results" section of this article (Dui et al., 2012).

Results:

2007: Mortimer and Moberg

Mortimer and Moberg were able to observe the tumor suppressive properties of *ago.* They found the protein is expressed in early embryos and that mutations of the protein can lead to embryonic death. Their data- with crosses of the *ago1* or *ago3* alleles produced mutant embryos that did not hatch. Their experiments indicated that *ago1* and *ago3* may be null or strong-loss-of-function alleles. (Mortimer, Moberg 2007). A null allele is one that has no role in gene product and a -loss-of-function allele is one that lacks the molecular function of the wild-type gene (National Cancer Institute).

They also noticed that defects in the mutant embryos are first seen in the trachea, which contains "breaks," or a lack of continuity in the tracheal lumen: these are visible in the dorsal trunk between dorsal branches of tracheal placodes. Their data revealed that an approximate 70% of *ago* mutant embryos had breaks in the dorsal trunk. They also noted a 15X increase in the rate of defective fusion events in mutants when compared with control embryos. When anti-B-gal staining of these embryos was performed, they found physical gaps between the cells of these tracheal placodes. This data suggests that *ago* is necessary to fuse these cells together and help the cells migrate within the trachea. Thus, they proved that *ago* DT breaks have to do with the failure of mutant DT fusion cells to fuse with placodes, further clearing up the misconception that *ago* loss in the DT relates to the actual number of cells.

Regarding their intent to study tracheal morphogenesis in *Drosophila*, they tested known *ago* target proteins to determine their ability to produce *ago* mutant tracheal phenotypes. They found that the expression of *cycE* and *dMyc* did not reproduce the mutant tracheal phenotype, which suggests that *ago* controls tracheal morphogenesis through another target. Furthermore, loss of function alleles like *trh* were able to dominantly suppress *ago* tracheal phenotypes. Their data suggested that *ago* may prevent Trh in the developing trachea. To test this, they stained wild type and *ago* mutant embryos with a Trh-specific antiserum. They found that *ago* mutant embryos had higher levels of Trh in tracheal cells. Thus, the final synopsis is that the interaction between *ago* and *trh* in the tracheal system stems from a requirement that *ago* must limit the levels of Trh in tracheal cells and specifically eliminate it from tracheal fusion cells.

Additionally, they found that the interaction between *ago* and the Trh target gene *btl* reveals that Trh-driven transcription of *btl* may correlate with the *ago* phenotype. *btl* expression patterns suggest that the failure to get rid of Trh in these cells results in ectopic *btl* transcription.

The lack of a similar effect in the remaining *ago* mutant tracheal cells suggests that more Trh must collaborate with fusion-cell specific factors to drive ectopic *btl* transcription.

Finally, they also found that *Ago* binds Trh and restricts Trh levels in cells. To examine this relationship, epitope tagged versions of the proteins were co-expressed in S2 cells. The results revealed that Trh accumulates to high levels in S2 cells but when co-expressed with Ago, it results in a suitable and reproducible reduction. When Ago and Trh are also expressed with Dys (which triggers Trh down-regulation in-vivo), the Trh levels drop significantly. This is consistent with findings that other F-box proteins are unstable when the concentration of their substrates vary. Overall, it is clear that Trh is a target of a pathway in embryonic tracheal cells and the mechanism in this effect requires *ago* to function and can be greatly amplified with a co-expression of *dys.*

Testing their hypothesis that *ago* and *trh* encoded products may interact in cells, the researchers found that Trh interacts with all three forms of Ago in the S2 cells they tested. Thus, they revealed that an *ago* mutation that does not regulate Trh levels in vivo cannot bind to the form of Trh that is needed for proteasome-dependent elimination in fusion cells. This proves that Ago and Trh have the ability to bind to one another, and this bond is needed to limit Trh levels in vivo (Mortimer, Moberg 2007).

Their results will be further analyzed in the “Discussion” section of this article.

2009: Mortimer and Moberg

In their 2009 experiment Mortimer and Moberg found that there are stage-specific effects of hypoxia on embryonic tracheogenesis. To test this, they placed wild type embryos in reduced oxygen environments. In the “early” embryos, they initiated hypoxic treatment at stage 11 when DT branches were actively migrating while the other “late” hypoxic treatment, which occurred at stage 15 occurred when DT fusion was already complete. After the treatments, the embryos were returned to normal O2 levels and developed to stage 16 when the scientists could see the tracheal architecture. Embryonic development was arrested by the stronger treatment but it resumed in levels where oxygen is normal, while the weaker hypoxia treatment only led to a slight delay in development. Early exposure to hypoxic environments led to stunted DT formation and fusion such that appeared unconnected. Structures like the lateral trunk, which form after fusion, were not as impacted. While the “early” treatment stunted growth, “late” exposure induced tube overgrowth. This data suggests that hypoxic activation will not always lead to overgrowth but it can also stunt growth if conducted within a specific time-period in embryonic development. The “early” system was less impacted by the effects when compared to the “late” system which is sensitized to the graded activation of the hypoxic response pathway (Mortimer, Moberg 2009).

They also discovered that dVHL is required to suppress the tracheal hypoxic response and that it genetically antagonizes *sima* in the embryonic trachea. To clarify, dVHL or *Drosophila* Von Hippel Lindau is a subunit of an oxygen-dependent ligase which degrades the SIMA-HIF 1α protein in animal cells. Then, they also shared data that proves that dVHl and *ago* work together to control embryonic tracheogenesis. Their observation that dVHL tracheal phenotypes require

sima but are only slightly sensitive to trh gene dosage suggests that *ago* and dVHL ubiquitin ligases prevent *btl* expression through distinct pathways. To test this theory, they examined the two types of dVHL embryonic tracheal phenotypes. In both cases, adding the *ago* allele changed frequent tracheal phenotype from overgrowing to stunted branch migration. Then, embryos with the ago3 allele and a deficiency that removes the dVHL locus showed a frequency of tracheal fusion and migration defects when put together. The lack of dVHL also enhanced the DT breaks, thus proving that dVHL and *ago* work together to control migration and fusing events. To test whether the interaction between dVHL and *ago* is specific to a certain developmental phase, the weaker dose of O2 was used to activate the dVHL/sima transcriptional program at the “early” or “late” time windows. With further analysis, they found that early exposure produces phenotypes at a low penetrance. However, reducing the dose of *ago* also led to a 3 times increase in the penetrance of the phenotypes. These effects included migration defects, duplicated secondary branches, and overgrowth. Thus, reducing the activity sensitizes the “early” system to changes in response to mild doses of hypoxia (Mortimer, Moberg 2009).

Their results will further be analyzed in the “Discussion” section of this article.

2012: Dui, Lu, Ma, and Jiao

Following their surveys, they revealed that there are 45 F-box genes in the *Drosophila* genome. While they analyzed several genes using RNAi analysis, for the sake of this article, data regarding the *Ago* gene will only be reported.

The scientists established in their surveys that *Ago* is encoded by CG15010 and that it targets Trh during trachea development, as well as CycE (promotes cell proliferation), dMyc (transcription factor regulating cell growth) and Notch (transmembrane receptor of Notch signaling). Overall, the greatest takeaway from this 2012 experiment is that the scientists identified *ago* as an F-box gene (Dui et al., 2012).

Their results will further be analyzed in the “Discussion” section of this article.

Discussion:

2007: Mortimer and Moberg

The researchers found that failure to degrade the target proteins promotes excess proliferation of imaginal disc cells. Their observations have led to the identification of Cyclin E and Myc proteins as targets of the *ago* gene. However, it is clear from their experiments that *Ago* is expressed in many areas, which suggests that it might have other processes and targets. Further research should look into identifying more of these other target proteins. This information will provide more insight into how *ago/Fbw7* are inactivated in cancers. Further research may be conducted to explore how a lack of *ago* function may impact tumorigenesis.

The researchers were able to clearly outline *Ago*’s role in tracheal morphogenesis. They also noted that *ago* mutant embryos first exhibited defects in their tracheal fusion cells. The DT break phenotype was incredibly high in these mutant embryos, and they found discrepancies in the rate of interplacode fusion. Their research is clearly well-thought out and well executed, but there was no evidence in their papers of multiple trials or a way for others to potentially repeat or

confirm their study. It is possible that in the future, further experiments can be conducted with other mutations of *ago* to also explore their effects on tracheal morphogenesis and dorsal trunk fusion.

One way to expand on the knowledge that the researchers collected in this study is by also considering the various other effects that concern tracheal morphogenesis. This is in-part what they did in 2009 with their study of hypoxic environments which will further be explored in this article. However, potential experiments that could sprout from this include discovering how mutations of *Fbw7*, the mammalian equivalent of *Ago*, may have similar effects in embryonic development.

2009: Mortimer and Moberg:

As a continuation of their 2007 experimental study of the *Ago* gene, these researchers were successful in identifying the effects of hypoxic environments on embryonic development. They found that embryos placed in hypoxic environments earlier on were better off in development than those placed in hypoxic environments during the later stages of embryonic development. The first genes that appeared to be impacted were tracheal genes, resulting in overgrowths and deformities. However, one limitation of the experiment overall is that the scientists did not look at an embryo's reaction to a hypoxic environment throughout its developmental process and instead only looked at how it affects development in two very specific time frames. This may be a potential way to further this experiment: by looking at how embryos placed in hypoxic environments throughout their growth stages are impacted. The scientists also discovered that hypoxia has a major impact on the primary and secondary trachela branches. On a more molecular level, they were able to conclude that *sima* and *btl* transcription by dVHL impact stunting and overgrowth in *Drosophila.* This demonstrates that dVHL has a significant role as an inhibitor of *sima* and *btl* when tracheogenesis occurs in normoxic environments.

Finally, one of their other discoveries was that dVHL also showed strong genetic interactions with alleles of the *ago* ubiquitin ligase subunit. This proves that *ago* also controls hypoxic sensitivity in the embryo. Their research highlights the possibility that there is a potential model in which each ligase acts through a target to regulate *btl* transcription in tracheal cells. This research can be applied to human research, as dVHl and *ago*'s mammalian equivalents are important tumor suppressor genes. Potential areas for further research could concern whether their ability to co-regulate tracheal morphogenesis is also found in mammals as well.

2012: Dui, Lu, Ma, and Jiao:

The intention of this article was to identify some of the other F-box genes in *Drosophila*, and the scientists were certainly successful at doing this: they found 45 F-Box genes. With the past two experiments, scientists were able to articulate how *Ago*, an F-box gene, was able to impact tracheal morphogenesis. One potential experiment that could be considered in the future is the study of how various F-box genes impact various systems.

The researchers also found various phenotypes associated with each gene and its specific RNAi line. This experiment is also highly reproducible and it is possible that other

scientists in the future could use the data collected by the scientists to study other F-box genes. Or, another future use for the data collected in this experiment is that it acts similar to a database for *Drosophila* F-box genes which scientists can use to access information while conducting their studies. Their data is connected by the common process, ubiquitination, which is relevant to various aspects of biology.

Overall, the intention of this review article was to articulate the impact of F-box genes in *Drosophila.* Through this article, by conducting an in-depth analysis of various experiments, this review synthesizes information regarding the function of F-box genes in *Drosophila.* Specifically, this review analyzed the Archipelago gene, which has been observed to have impacts on tracheal morphogenesis and dorsal trunk fusion in early embryonic development in *Drosophila.* Research regarding the experiments conducted with the Archipelago gene has come to a conclusion, as the last paper studying these genes was published in 2012. However, this review could serve as a resource for those who would like to understand the Archipelago gene and its impact on *Drosophila* in early embryonic development. Additionally, few have researched the implications of the Archipelago gene equivalent in humans– FBW 7. As cancer research continues, we continue to remain hopeful that the research on F-box genes in *Drosophila* may prove to be useful in studying mammalian disease and genetic development as well.

References

1. Tolwinski N. S. (2017). Introduction: Drosophila-A Model System for Developmental Biology. *Journal of developmental biology*, *5*(3), 9. https://doi.org/10.3390/jdb5030009
2. Papagiannouli, F., & M., B. (2013). Modeling Tumorigenesis in Drosophila: Current Advances and Future Perspectives. InTech. doi: 10.5772/55686
3. *Facts*. Facts - Fruit Flies | Max-Planck-Gesellschaft. (n.d.). https://www.mpg.de/10973625/why-do-scientists-investigate-fruit-flies#:~:text=Outwardly%2C%20fruit%20flies%20and%20humans,humans%20in%20a%20similar%20form.
4. Tolwinski N. S. (2017). Introduction: Drosophila-A Model System for Developmental Biology. *Journal of developmental biology*, *5*(3), 9. https://doi.org/10.3390/jdb5030009
5. Medina, I., Calleja, M., & Morata, G. (2021). Tumorigenesis and cell competition in *Drosophila* in the absence of *polyhomeotic* function. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(45), e2110062118. https://doi.org/10.1073/pnas.2110062118
6. Herranz, H., & Cohen, S. M. (2017). Drosophila as a Model to Study the Link between Metabolism and Cancer. *Journal of developmental biology*, *5*(4), 15. https://doi.org/10.3390/jdb5040015
7. Archipelago. (n.d.). https://www.sdbonline.org/sites/fly/sturtevant/archipelago1.htm
8. Welcker, M., & Clurman, B. E. (n.d.). *FBW7 ubiquitin ligase: A tumour suppressor at the crossroads of cell division, growth and differentiation*. Nature News. https://www.nature.com/articles/nrc2290
9. Chung, S., Chavez, C., & Andrew, D. J. (2011). Trachealess (Trh) regulates all tracheal genes during Drosophila embryogenesis. Developmental biology, 360(1), 160–172. https://doi.org/10.1016/j.ydbio.2011.09.014
10. Jiang, L., & Crews, S. T. (2003). The Drosophila dysfusion basic helix-loop-helix (bHLH)-PAS gene controls tracheal fusion and levels of the trachealess bHLH-PAS protein. *Molecular and cellular biology*, *23*(16), 5625–5637. https://doi.org/10.1128/MCB.23.16.5625-5637.2003
11. Mortimer, N. T., & Moberg, K. H. (2007). The Drosophila F-box protein Archipelago controls levels of the Trachealess transcription factor in the embryonic tracheal system. *Developmental biology*, *312*(2), 560–571. https://doi.org/10.1016/j.ydbio.2007.10.002
12. Mishra, P., Singh, U., Pandey, C. M., Mishra, P., & Pandey, G. (2019). Application of student's *t*-test, analysis of variance, and covariance. *Annals of cardiac anaesthesia*, *22*(4), 407–411. https://doi.org/10.4103/aca.ACA_94_19
13. Mortimer, N. T., & Moberg, K. H. (2007). The Drosophila F-box protein Archipelago controls levels of the Trachealess transcription factor in the embryonic tracheal system. *Developmental biology*, *312*(2), 560–571. https://doi.org/10.1016/j.ydbio.2007.10.002
14. Mortimer, N. T., & Moberg, K. H. (2007). The Drosophila F-box protein Archipelago controls levels of the Trachealess transcription factor in the embryonic tracheal system. *Developmental biology*, *312*(2), 560–571. https://doi.org/10.1016/j.ydbio.2007.10.002

15. Mortimer, N. T., & Moberg, K. H. (2009). Regulation of Drosophila embryonic tracheogenesis by dVHL and hypoxia. *Developmental biology*, *329*(2), 294–305. https://doi.org/10.1016/j.ydbio.2009.03.001
16. Dui, W., Lu, W., Ma, J., & Jiao, R. (2012). A systematic phenotypic screen of F-box genes through a tissue-specific RNAi-based approach in Drosophila. *Journal of genetics and genomics = Yi chuan xue bao*, *39*(8), 397–413. https://doi.org/10.1016/j.jgg.2012.05.009
17. Dui, W., Lu, W., Ma, J., & Jiao, R. (2012). A systematic phenotypic screen of F-box genes through a tissue-specific RNAi-based approach in Drosophila. *Journal of genetics and genomics = Yi chuan xue bao*, *39*(8), 397–413. https://doi.org/10.1016/j.jgg.2012.05.009
18. Mortimer, N. T., & Moberg, K. H. (2007). The Drosophila F-box protein Archipelago controls levels of the Trachealess transcription factor in the embryonic tracheal system. *Developmental biology*, *312*(2), 560–571. https://doi.org/10.1016/j.ydbio.2007.10.002
19. *NCI Dictionary of Genetics terms*. National Cancer Institute. (n.d.). https://www.cancer.gov/publications/dictionaries/genetics-dictionary
20. Mortimer, N. T., & Moberg, K. H. (2007). The Drosophila F-box protein Archipelago controls levels of the Trachealess transcription factor in the embryonic tracheal system. *Developmental biology*, *312*(2), 560–571. https://doi.org/10.1016/j.ydbio.2007.10.002
21. Mortimer, N. T., & Moberg, K. H. (2009). Regulation of Drosophila embryonic tracheogenesis by dVHL and hypoxia. *Developmental biology*, *329*(2), 294–305. https://doi.org/10.1016/j.ydbio.2009.03.001
22. Mortimer, N. T., & Moberg, K. H. (2009). Regulation of Drosophila embryonic tracheogenesis by dVHL and hypoxia. *Developmental biology*, *329*(2), 294–305. https://doi.org/10.1016/j.ydbio.2009.03.001
23. Dui, W., Lu, W., Ma, J., & Jiao, R. (2012). A systematic phenotypic screen of F-box genes through a tissue-specific RNAi-based approach in Drosophila. *Journal of genetics and genomics = Yi chuan xue bao*, *39*(8), 397–413. https://doi.org/10.1016/j.jgg.2012.05.009