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The Promises of Neurodegenerative Disease Modeling

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The promises of neurodegenerative disease modeling

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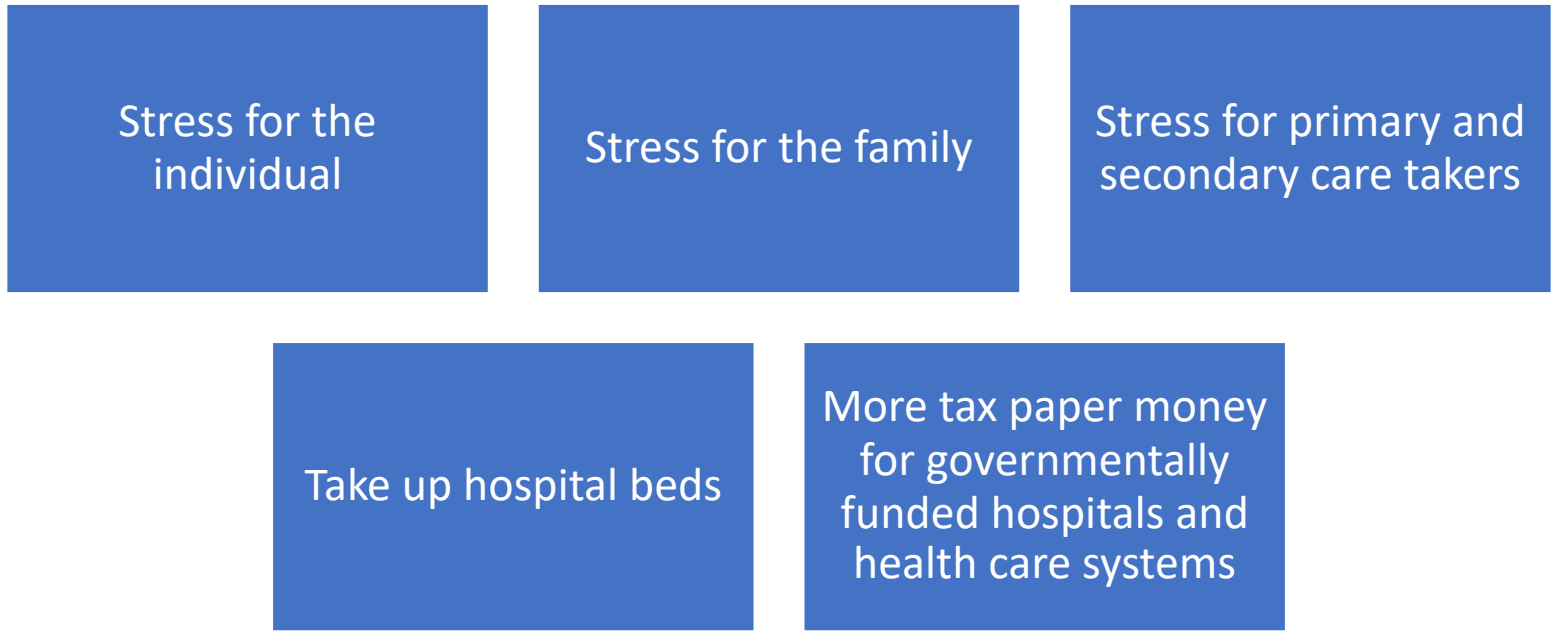
The purpose of the study

- With the rise of the human lifespan has come the rise of age-related disorders, particularly neurodegenerative diseases that express themselves late in life such as Alzheimer's disease (AD) and Parkinson's disease (PD)
  - However, age-related diseases also means more research spending in cancer treatments
- The paper seeks to (1) evaluate the role of molecular genetics in research studies and (2) examine the use of *D. melanogaster* (the common fruit fly)

Introduction

- Medical problems such as Parkinson's disease pose a problem for both the individuals diagnosed with the diseases and those who have to take care of the affected individual



Introduction

- These stressors necessitate government involvement through research.
  - These research programs take up money that could be spent toward other programs such as Social Security and employee benefits
  - The private sector could be delegated the task of conducting research
  - However, this may mean fewer treatments for patients and less novelty in daily life
- Discussion question: Regarding research, are you happy with the government's allocation of money? Should the government change the amount of spending it is putting towards research?

Introduction

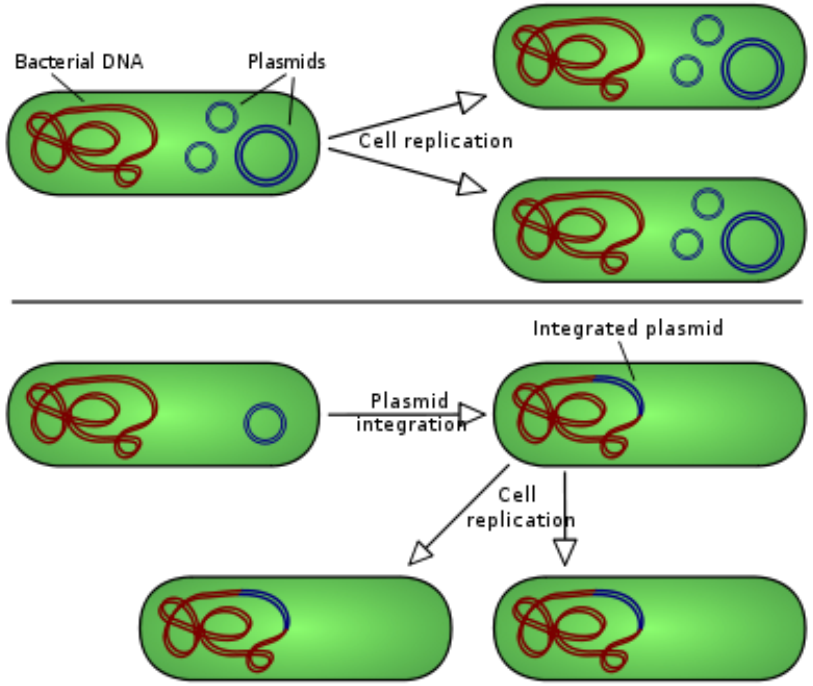
- Before testing on human subjects, research is largely conducted on less-expensive and lower-risk species such as mice that mimic the human genome
- However, genetic engineering has also allowed us to edit the genomes of these species and mimic the conditions in individuals with altered genes (like in individuals with genetically-linked diseases)

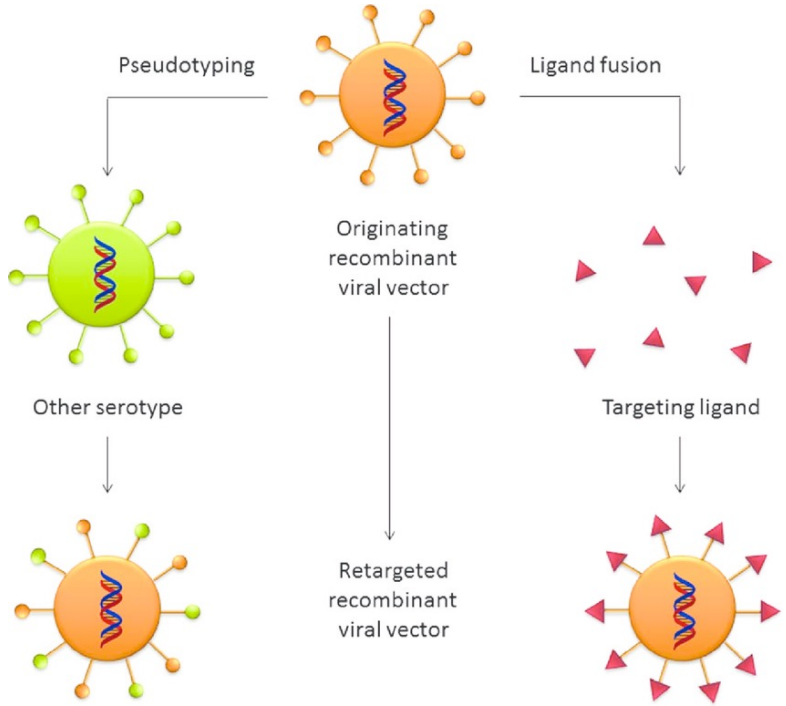
Molecular genetics and the diversification of animal model organisms

- Besides the costs and ethical dilemmas, there are two main reasons why using animals with germ lines similar to those of humans is useful
  - When the genome of these species is unaltered, they allow us to test whether various chemicals or treatments are useful to regular people
  - When their genomes are edited, they allow us to mimic patients with preexisting conditions
- The latter has been made possible through genetic engineering

Molecular genetics and the diversification of animal model organisms

- Method 1: The use of recombinant DNA to splice DNA fragments into bacteria using plasmids and viral vectors





Molecular genetics and the diversification of animal model organisms

- Bacteria are single-celled organisms
- Method 2: The introduction of genetic engineering into single-celled species paved the way for the introduction of genetic engineering into multicellular organisms and ultimately humans
- The transgenic approach was introduced into mice in the early 1980s and eventually fruit flies later on
- These animal models have been instrumental in studying the neurodegenerative effects of Alzheimer’s, Parkinson’s, ALS, and Huntington’s disease

Molecular genetics and the diversification of animal model organisms

- Method 3: Genome sequencing allowed researchers to take a look at the genomes of various animals species such as nematodes and mice
- This allows researchers to determine animal counterparts to humans that can fit within their realm of study
- Genome sequencing also reveals the degree to which genes have been preserved over the course of evolution
  - Not only is the structure of the resulting proteins the same but also their function in those organisms
- This further affirms the use of animal models as human alternatives

Molecular genetics and the diversification of animal model organisms

- Method 4: Come up with DNA probes that can quantify the expression of certain genes in different organisms
- Even though we may share many of the same genes as mice (99% similarity), mice definitely express different genes that we do, leading to their different physiology and responses to treatments
- What this means is that animals cannot be the end-all-be-all of drug testing: human trials are required

Advantages and purpose of simpler organisms

- Simpler human alternatives have the following advantages:
  - They are genetically similar to humans
  - They do not pose as many ethical dilemmas (especially simpler organisms)
  - They are less expensive to maintain
  - Animals reproduce more often
  - They produce more offspring
  - They are more freely available
- However, animal models also have various disadvantages

Advantages and purpose of simpler organisms

- Disadvantages of animal models
  - They do not perfectly mirror the human genome
  - Some species (such as pigs) still pose ethical dilemmas
  - At a large scale, high costs up-front
  - High personal effort on the part of researchers (mice will not maintain themselves)
  - The integration of animal models in research means that there are more barriers in introducing a new treatment to the public
- What side does the author err on?
  - The author would suggest that the advantages outweigh the disadvantages
  - With similar genomes, similar responses to treatments are ensured
  - Animal testing is required for human approval
  - They provide more in-depth insight as you can cut them up and observe them without ethical issues

Drosophila melanogaster as a model organism of choice to study neurodegenerative diseases

- So, animal models are effective
- Now, what animal should you use for neurodegenerative disorders?
- Mice have complex nervous systems, but they can pose ethical dilemmas and cost more to maintain and test on
- *D. melanogaster* has nerve cells exposed (eye), but they are difficult observe *in vivo*
- Nematodes are simple organisms, but they have primitive nervous systems

Drosophila melanogaster as a model organism of choice to study neurodegenerative diseases

Mouse

- Most expensive to maintain and examine
- Difficult to manage
- Pose ethical dilemmas
- Complex nervous systems (70 million neurons)

Fruit Fly

- Easy to maintain
- Eyes can be examined for neurodegeneration
- Do not pose ethical dilemmas
- Relatively simple nervous systems (200,000 neurons)

Nematode

- Easy to maintain and purchase
- Easy to genetically modify
- Do not pose ethical dilemmas
- Very simple nervous systems (302 neurons)

Drosophila melanogaster as a model organism of choice to study neurodegenerative diseases

- Upon other advantages, *D. melanogaster* has several benefits over other organisms in modeling neurodegenerative diseases
  - Easy to reproduce
  - Many offspring
  - ”At least 77% of the human disease-associated genes reported in the Online Mendelian Inheritance in man database have evolutionary similarities with Drosophila genes”

Drosophila and the study of neurodegeneration

- Fruit flies have been used for over 100 years in biological research, so they have a proven track record for use in research
- Benzer et al. conducted the first studies with fruit flies
- Since then, 4 approaches have been developed to study fruit flies in regard to neurodegeneration

Drosophila and the study of neurodegeneration

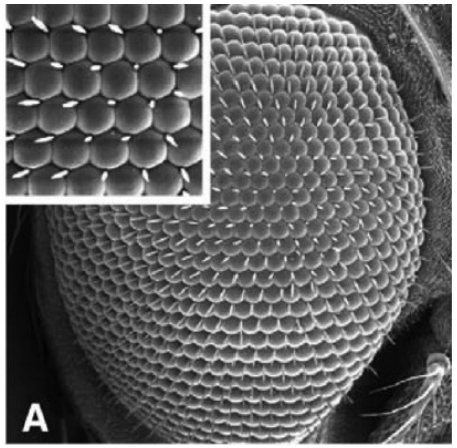
- Method 1: Isolating genes and mutations that lead to neurodegeneration (“forward screens”)
- Change up the phenotype (use different individuals) and observe which gene was making the difference
- RNA interference (RNAi) has allowed researchers to disrupt *D. melanogaster* genomes and observe which genes lead to maladaptive effects

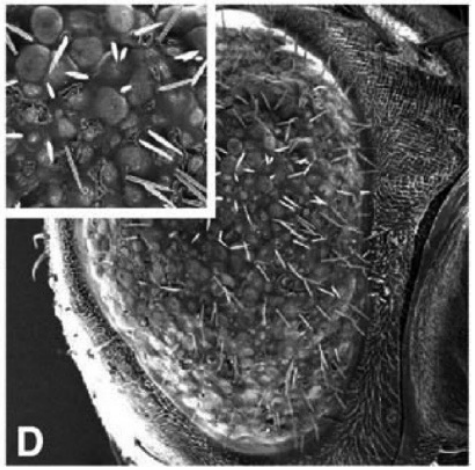
Drosophila and the study of neurodegeneration

- Method 2: ”reverse screens”
- Alter the genotype, and observe the phenotype
- CRISPR/Cas9 can be used to edit the genome on a large scale with extreme precision
- This would only be ethical in simple organisms, however
- Discussion Question: Do you think that reverse screens would be ethical in humans? What about the prospects of CRISPR/Cas9 serving as a tool for making designer babies?

Drosophila and the study of neurodegeneration

- Method 3: Studying the fly's eye
- *D. melanogaster* has a large, repetitive visual receptor array influenced by the health of feeding neurons
- If the visual receptors are altered, they can be observed and a "rough" appearance to the eye is common





Drosophila and the study of neurodegeneration

- Method 4: Using enhancer-suppressor modifier genes
- Some genes are directly linked to certain phenotypes such as nerve toxicity
- However, other genes control the expression of the mutated genes, leading to higher or lower expression
- Altering or removing the modifier gene can increase or decrease the severity of a condition, or make the expression of the first gene out of control
- Inference: fighting tumorigenic modifier genes could serve as a disease-modifying treatment for cancer

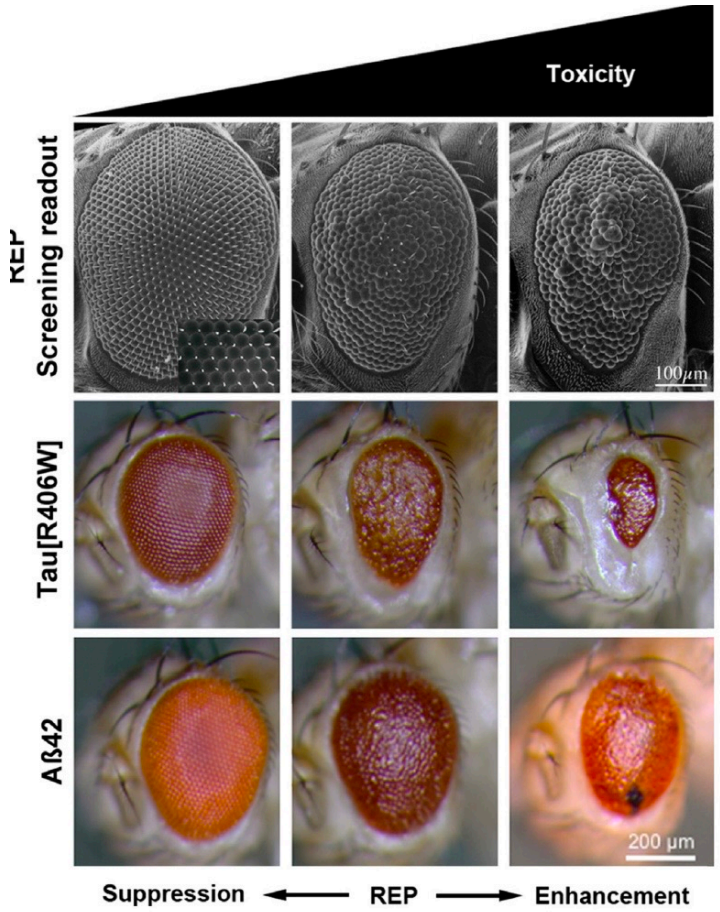
What has been found using Drosophila?

- Forward screens of the fruit fly genome have found over 40 genes to the “recessive loss of functions” (like AD and PD)
- More than half of these genes have been found to be linked to neurodegeneration
- “This list underestimates the actual number of genes whose mutation may lead to a loss of integrity or function of the fly nervous system.”
- Evidence has shown that mutations of the *parkin* and *pink1* genes are associated with PD

What has been found using Drosophila?

- One functional screen on the *D. melanogaster* genome has examined 87 loss-of-function genes linked to Alzheimer's and found several modifier genes, including some that alter the presence of the Tau protein (leads to neurodegeneration through the amyloid-β peptide)
- *In vivo* studies in *D. melanogaster* have shown a linkage in APP (a precursor to the amyloid protein) and neuroanatomical defects linked to Alzheimer’s
- Recent news: Aducanumab has recently been approved as a treatment for Alzheimer’s (June 7th)

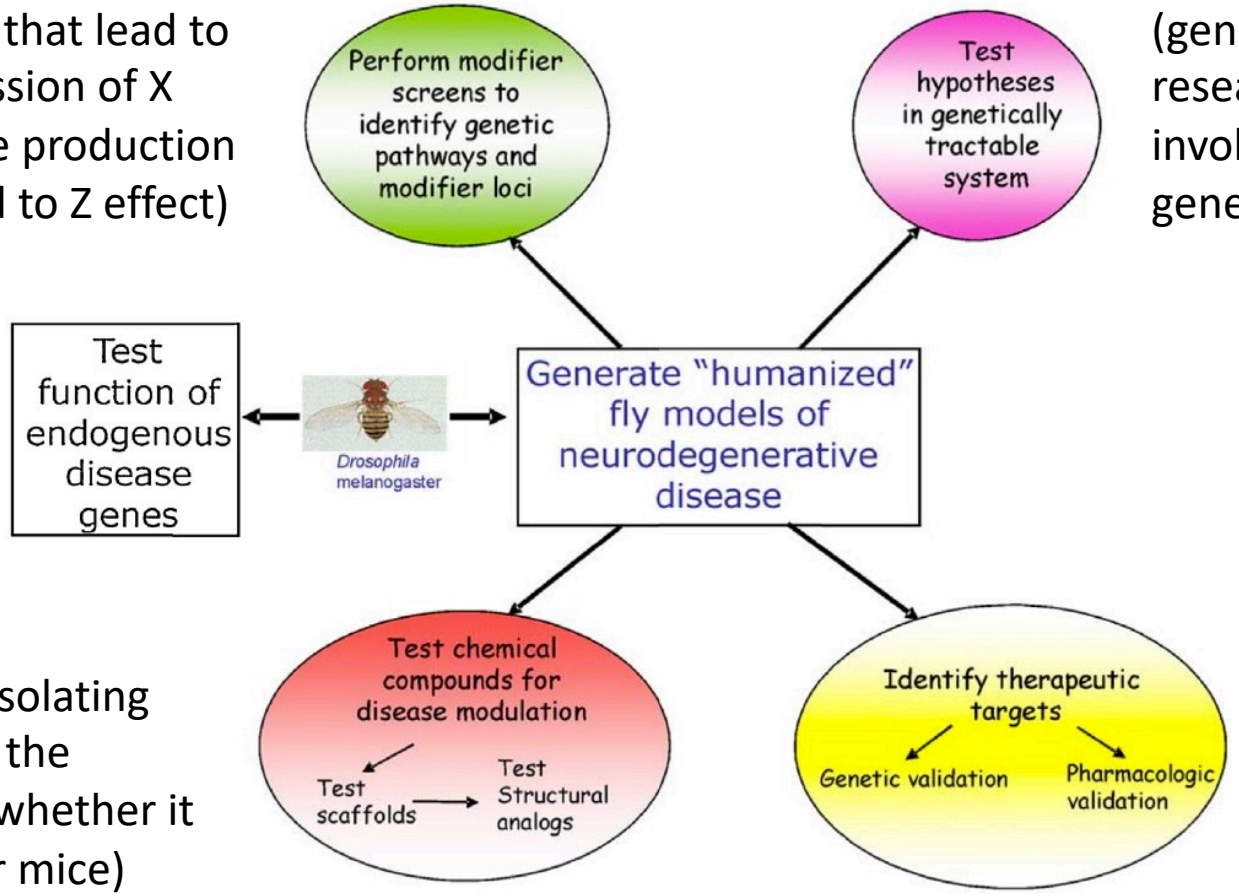
What has been found using Drosophila?



- Fruit flies have also been used to identify that exon 1 of the Huntington's gene is particularly pathogenic, making a potential target for therapy
- Similar neurodegeneration as shown on the left can be expected from the Huntington's gene

Future prospects for the fly model

(identify pathways that lead to disease: the expression of X gene leading to the production of Y protein to lead to Z effect)



(generation of basic and applied research designs to test the involvement of the identified gene in neurodegeneration)

(potential application is isolating the protein coming from the identified gene and test whether it has effects on humans or mice)

(identify genetic targets for the development of drugs designed to deter the expression of the gene in clinical studies)

Future prospects for the fly model

- My interpretation of the diagram prior to this slide
  - First the genetic origin of a particular phenotype or disease can be tested in *D. melanogaster*. Protective substances can also be tested for effectiveness against neurodegeneration
  - Secondly, studies in higher-order, mammalian organisms such as mice or rats can be conducted
  - Lastly, human trials can be conducted for effectiveness

Future prospects for the fly model

- Although the fly model most definitely is useful, it is not perfect
  - Compared to humans, flies have relatively simple nervous systems, meaning they are not dependable for direct translation into human research (mice generally serve as the middle ground)
  - Flies are arthropods while humans are vertebrates, marking a divergence in evolution. Thus, the disease-linked genes in flies may not be causative factors for neurodegeneration in humans

Future prospects of the fly model

- Although *D. melanogaster* differs from humans, it has a number of benefits still
  - Its genome has been thoroughly mapped
  - The protective role of various chemicals can be examined
    - Lithium use in fruit flies has been shown to suppress the accumulation of amyloid-β in the Alzheimer’s model
    - Methylene blue (a common staining tool) has been shown to lower neurodegeneration in Huntington's’ patients
    - A short peptide from Huttingtin has been shown to decrease the accumulation of the polyglutamine protein in the fly HD model
  - It is the best-analyzed and best understood multicellular organism to date

Summary

- Molecular genetics has played a powerful role in allowing us to discover the effects of genetic mutations and conditions, as well as identify animal models for treatment development in humans
- *D. melanogaster* serves as a promising human alternative for testing in research trials

Discussion Questions

- Going back to the very reason for this study, diseases present themselves late in life. While many are unavoidable (such as HD Type II diabetes), many are avoidable. Should the government spend more money trying to encourage healthy living habits or fund research to produce treatments for avoidable diseases?
- Do you think that *D. melanogaster* serves as an adequate alternative to human *in vivo* trials? Would mice models suffice in their place?
- One of the foundational tools in the review was the use of molecular genetics. Do you think neurodegenerative diseases can be explained in light of evolution (genetics), or are the result of the present (modern medicine)?

Discussion Questions

- What do you think about the use of animal models in general? Is there an alternative way to coming up with drugs and treatments that does not involve harming animals?
- Based upon the findings in the review, what do you think would be some good follow-up experiments that would benefit the larger scientific community?

Citation

- Lepesant, J. (2015). The promises of neurodegenerative disease modeling. Comptes Rendus Biologies, 338(8-9), 584-592. doi:10.1016/j.crvi.2015.06.018