Julie Grandsire
AgroParisTech
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Model organisms in molecular biology

Seminar

Fruit Fly : a model organism for neurodegenerative diseases

Slide n°2
First of all, it's important to remind us what is a neurodegenerative disease (NDD). According to definitions, it's a « loss and death of neurons and functions of the human brain ». This type of disease can be caused by genetic factors as well as environmental factors. Indeed, some genes can make a person more at risk for developping a NDD and then the severity of the disease depends on the environmental exposure during the person's life [1]. After that, disruptions can lead to several symptoms, which depend on the disease, but can be physical, behavioural or even cognitive limitations [4]. Today, NDD affect millions of people worldwide and the risk increases with the age. Unfortunately, we don't have treatments to cure these diseases yet... We just have treatments to help relieve some physical and mental symptoms which is already a good progress but it's not enough because it doesn't slow the disease progression. As our life expectancy is increasing, the number of people with this type of disease is increasing too, that's why we need more than never to focus on this worldwide health issue [1]. Moreover, NDD affect not only aged people but also infants and children [5]. We really need to improve our understanding about the causes of NDD (genetic and environmental), the pathogenic mechanisms, etc, in order to prevent these diseases and to find treatments which cure them [1].

Slide n°3
Today, we all have heard about NDD. The most common are Alzheimer's disease (AD) and Parkinson's disease (PD). For instance, in 2016, 5.4 million Americans had AD and the estimations for PD in 2020 were about 930,000 Americans [1]. In the United States, AD is the sixth leading cause of death [5].
One common feature for all these NDD is that there is an abnormal accumulation of misfolded protein aggregates. For example, in AD, it's because of amyloid beta (Aβ) and tau protein aggregates. For PD, it's because of α-synuclein protein aggregates. For Huntington's disease (HD), it's because of HTT protein aggregates, and for Amyotrophic Lateral Sclerosis (ALS), protein aggregates are made of FUS, TDP-43 and SOD-1. These aggregates become neurotoxic and perturb vital cellular processes, causing loss of synapses and neurons [4].

Slide n°4
Fruit Fly, also called *Drosophila melanogaster,* is an insect coming from Western Africa. Today, it's spread all over the world, present in every continent, in every country. It's one of the first organisms which is use as a model for other organisms like humans. Indeed, it was used a lot from the Ancient Greece, up to 1940. Today, it's genome is completely sequenced (since 2000) and scientists created one of the first database thanks to that.

Slide n°5
But we can wonder : Why is fruit fly a model organism for humans ?
Fruit fly was one of the organisms chosen as a model for humans because it has many advantages. Indeed, to name a few, fruit fly has a small genome size which enable scientists to focus on a little number of genes, to detect mutations easily and to manipulate it with more facility. Plus, fruit fly

has a short generation time which allows scientists not to loose time between the experiments, to observe the effects of the mutations on individuals of the next generation quickly etc. Moreover, the number of progeny is very high, with about 100 individuals. This organism also has a low genetic redundancy which is a good thing to study only the main part of the genome. Another advantage among others is the availability of tools for genetic manipulation. All these advantages allow us to use fruit fly easily for lots of types of experiments.
In an interview, Professor Charalambos Kyriacou (University of Leicester) said « We know where every gene is, where every chromosome is, we can put gene in to drosophila, we can put gene out to drosophila, we can knock down all genes, we can mutate every gene you like, in every tissue you like, so it's the organism par excellence in genetic research » [2]. This shows that we can really do whatever we want with fruit flies.
Last but not least, about 75% of the human disease genes have fly homologs, which means that *Drosophila* is an extremely valuable system to identify and validate the biological roles of new disease-associated genes [4].
That's why, nowadays, fruit fly is a model for developmental diseases, neurological, tumors, heart diseases and also for drug development.

Slide n°6
But why is fruit fly a model specifically for NDD ?
At the beginning, research about NDD were done on yeasts. Yeasts are also model organisms for studying humans issues and for example, in the case of the HD, scientists managed to identify responsible genes and proteins for this disease in yeasts. This was a first breakthrough but then, scientists were blocked because they couldn't observe the effects of the mutated genes on the phenotype. Indeed, a yeast is a unicellular organism whereas humans are multicellulars. Plus, yeasts don't have a neurval system contrary to humans [2]. These two facts made them use an other model organism : *Drosophila melanogaster*, because this one is multicellular and has a neurval system. They took the candidate genes found in yeasts and then did experiments in these genes in fruit flies. They observed the same neurodegeneration as for human patients, namely neurone loss, impairment of locomotor activity, reduced survival etc [3]. So the use of fruit fly enables the exploration of the mechanisms of neuronal function, survival and degeneration *in vivo*.
We can link these discoveries to humans because there are lots of similarities in the nervous system function and organization of these two organisms. Indeed, they have lots of homolog genes involved in NDD and fruit flies develop neurodegenerative phenotypes that are very similar to humans [4]. That's why various NDD have been modelled and studied successfully in flies such as PD, AD, HD and ALS.

Slide n°7
In order to detect the genes responsible for NDD, researchers use genetic tools to do genetic screens. The 2 major echniques used are the Forward genetic screens and the Reverse genetic screens. I'm going to explain these techniques in the following slides but both of them ultimately allow us to understand complex mechanisms that are involved in neuroprotection and neurodegeneration.

Slide n°8
The forward genetic screen consists in introducing lots of mutations in the whole genome of the organism. Then, it leads to generation of flies with some normal individuals and some other individuals with aberrant phenotypes. The flies with interesting phenotype, that is to say with the phenotype of the NDD, are taken and their genome is mapped. This whole process enables the discovery of genes involved in the studied NDD. The last step is to determine human homologs for these responsible genes. To be sure that the human homolog gene is also responsible for NDD in humans, scientists can express cDNA of this gene in the corresponding fly mutant to test the conservation of gene function between fly and human, and to confirm its disease causation [4].

An example of the use of this method is with the Neurometabolic disease ARSAL. Scientists put mutations in the whole genome of flies. Amoug the progeny, they observed some individuals with symptoms of this disease like retinal and muscle degeneration, impaired cell proliferation etc. Thus, they mapped their genomes and found the responsible gene: *Aats-met* gene (which encodes the mitochondrial methionine-tRNA synthetase). The human homolog of this gene is MARS2 and to be sure the mutation of this gene is also responsible for ARSAL in humans, they did the rescue experiments with cDNA as explained before and it was a success [4].

Slide n°9
Reverse genetic screens is different because we already know the gene we want to mutate. Thus, we target the gene and mutate it. Then, we wait and we can observe the phenotype caused by the mutation of the known gene [4].
We can also study the post-developmental function of genes that are essential for development and survival thanks to another method which is RNAi screens. Indeed, if we mutate the genome at the beginning as for the other methods, we can see the effects on the progeny but then they will die because the phenotype and the disease are not viables. Because of that, we can't see if there would have occurred other problems after in their life. That's why they use this RNAi method which consists in adding a mutation in the genome but whenever we want, at every time in the organism life [4].
On the whole, genetic screens has helped in the discovery of new players in neurodegeneration.

Slide n°10
The first part of my presentation was made thanks to several documents. Now, we'll see all of that thanks to a precise example taken from a paper : Deal SL and Yamamoto S (2019) Unraveling Novel Mechanisms of Neurodegeneration Through a Large-Scale Forward Genetic Screen in Drosophila. Front. Genet. 9:700. doi: 10.3389/fgene.2018.00700.
The objective of this study is the identification of a number of essential genes that exhibit neurodegenerative phenotypes when mutated thanks to genetic screens on the fly X-chromosome.

Slide n°11
First of all, scientists tried to identify essential genes on the fly X-chromosome that are required for neuronal maintenance. This first part was based on the following observation : “Based on information from FlyBase, 290 X-linked genes have been associated with a lethal mutation to date, suggesting that there are a significant number of essential genes on this chromosome”. In order to discover those genes, they designed and performed a three generation (F3) screen using EMS (ethyl methanesulfonate) mutagenesis and FLP/FRT mediated mitotic recombination. The combination of these techniques enabled the creation of mosaic fruit flies, that is to say flies whose eyes are made of two types of cells. Red cells (w+) are transheterozygous for the mutant of interest, and behave as control cells. White cells (w-) are homozygous for the mutant of interest.

Slide n°12
Once mosaic flies were produced, they aged them for 3 weeks after eclosion and recorded the electroretinograms (ERG) of homozygous mutant patches of cells in order to record the photoreceptor and post-synaptic neurons activities. This method enables researchers to detect defaults of these functions thanks to the shapes of the curves in the ERG. To do that, they placed a field recording electrode on the mutant region (white patches), inserted a reference electrode in the thorax, and shone a white light for 1s. Abnormal traces of depolarization or repolarization (2&4) suggest defects in phototransduction or overall integrity of the photoreceptor, whereas alterations in on- and off-transients (1&3) suggest problems with synapse formation or function. Thanks to that, they can detect genes required for neuronal maintenance in the X-chromosome. Moreover, 93% of the genes from the screen have a human homolog so it can explain the origin of some human NDD.

Slide n°13
Additional experiments can be done to caracterize a little bit more the phenomenon.
In the study, they used the Transmission Electron Microscopy (TEM). They did cross sections of several parts of the eyes as ommatidia or lamina cartridge and then observed them with TEM. This technique can give indicators of cellular health and determine if there are structural deficits in newly eclosed flies and thus can detect any pre symptomatic signs of neurodegeneration.
They also did functional assays specific to the gene of interest which enable the understanding of the molecular mechanisms underlying neurodegenerative phenotypes. They found that many of the mutants were in genes functionally related to the mitochondria...

Slide n°14
Here you can see the essential genes required for neuronal maintenance that they found in this study (in blue). For instance, there are Vps26, Crag, Nrd1 and Marf, which we are going to discuss about.

Slide n°15
Neurons are highly metabolically active cells and thus, they need huge amount of energy in the form of ATP for their functions and survival. The major part of ATP production takes place in the mitochondria. If mitochondria don't function well, it leads to NDD. Indeed, mutations in mitochondrial proteins or genes can be involved in activation of more than one pathological mechanisms, ultimately leading to neurodegeneration. We know that many mitochondrial diseases exhibit neurodegenerative phenotypes, and mitochondrial dysfunction is one of the main feature associated with neurological disorders such as Alzheimer's disease, Parkinson disease and Amyotrophic Lateral Sclerosis. Moreover, most genes that function in mitochondria are conserved between humans and Drosophila, allowing exploration of their in vivo functions in flies.
In the following slide, we'll focus on 1 gene in mitochondria.

Slide n°16
Marf, Mitochondrial assembly regulatory factor, is a GTPase. When this gene is mutated, it leads to several consequences. It reduces the energy production so the production of ATP which is necessary for neurons. It also blocks the mitochondrial trafficking down the motor neuron axons and futhermore it produces defects in synaptic morphology at the neuromuscular junction because of the lack of mitochondrial-ER tethering. We can assess that Marf homologs in human are both MFN1 and MFN2. In order to check this fact, scientists did experiments : they tried to rescue the loss of Marf in Drosophila thanks to the MFN1 and MFN2. They found that MFN2 was sufficient to rescue the ER tethering, but both MFN1 and MFN2 were required to fully rescue the synaptic morphology. Thus, when mutated, MFN1 and MFN2 are responsible for NDD like Charcot-Marie-Tooth disease in human.

Slide n°17
Conclusion
Fruit flies are a good model thanks to all the advantages of this organism. Thus, we can do experiments on them and then transpose the discovering to humans. Indeed, many parallels can be drawn between Drosophila and human phenotypes, suggesting that most mechanisms underlying demise of neurons are evolutionarily conserved.
In the last study I mentioned, they noticed that 93% of the genes from the X-chromosome screen have human homologs. Considering the importance of these genes to survival and nervous system function in Drosophila, they predict that most, if not all, conserved X-screen genes will eventually be linked to human diseases.
Besides, various NDD have been modelled and studied successfully in flies such as PD, AD, HD and ALS.

Plus, we have to keep in mind that *Drosophila* can also be used to perform drug screenings. "By discovering new genes required for neural maintenance in flies and working with clinicians to identify patients with deleterious variants in the orthologous human genes, Drosophila biologists can play an active role in personalized medicine." [5].

References

[1] https://www.niehs.nih.gov/research/supported/health/neurodegenerative/index.cfm

[2] https://www.youtube.com/watch?v=Jj5QlYlE66w

[3] https://www.youtube.com/watch?v=CrvK0dLhChw

[4] NEHA SINGHAL and MANISH JAISWAL (2017) Pathways to neurodegeneration: lessons learnt from unbiased genetic screens in *Drosophila*

[5] *Deal SL and Yamamoto S (2019)* Unraveling Novel Mechanisms of Neurodegeneration Through a Large-Scale Forward Genetic Screen in Drosophila. Front. Genet. 9:700. doi: 10.3389/fgene.2018.00700