Fruit fly as a model for inherited heart diseases

To deal with this subject, I studied the review: “Drosophila in the Heart of Understanding Cardiac Diseases : Modeling Channelopathies and Cardiomyopathies in the Fruitfly”. This review was written by Ouarda Taghli-Lamallem, Emilie Plantié and Krzysztof Jagla. It was published on 18 February 2016 in the Journal of Cardiovascular Development and Disease.

First, I will introduce a few concepts about Fruitfly. It is known by the scientific name of *Drosophila melanogaster.* Its genome was fully sequenced in 2000 and therefore includes 180 Mb and 13500 genes. We will see that there is a significant conservation of the genome between *Drosophila melanogaster* and humans. This organism is one of the most studied in biological research because it has many advantages. Indeed, this species has a short generation time (about 10 days), it is very simple to breed in the laboratory because it does not require much equipment and space. Also, it has a high fertility (females can lay up to 100 eggs per day). One of its genetic advantages is the fact that it is easy to perform crossings. Fruitfly is used as a genetic model in several human diseases such as: neurodegenerative diseases, endocrine diseases and heart diseases. These are the ones that will interest us.

For this seminar, the first phase will be devoted to a general introduction and an explanation of the concepts. The second part will focus on Fruitfly's contribution to the understanding of channelopathies and cardiomyopathies. Finally, a last part will highlight the pathological mechanisms involved in the cardiac diseases mentioned and the challenges posed by Fruitfly as a model organism.

1. Introduction

Channelopathies designate all the diseases related to a dysfunction of the membrane ion channels. They are responsible for heart rhythm and muscle contraction disorders. As for cardiomyopathies, it is a group of diseases responsible for myocardial dysfunction. Cardiomyopathies can have an extrinsic or intrinsic origin. Cardiomyopathies can be asymptomatic, but they can also be responsible for asthenia (weakening of the body), dyspnea (shortness of breath), rarely chest pain, tachycardia and other heart rhythm disorders. Cardiovascular diseases (CVD) remain the leading cause of death worldwide, with 46% of all deaths in Europe and 31.9% of all deaths in the United States. There are three types of cardiomyopathies:

- Hypertrophic (HCM). This is the most common (1/500 ind). Here are examples of symptoms: increased thickness of the wall of the left ventricle, myocytic hypertrophy...
- Dilated (DCM) (1/2500 ind) Symptoms are: systolic dysfunction, which leads to progressive heart failure and sudden cardiac death due to ventricular arrhythmia.
- arrhythmogenic right ventricular (ARVC) (1/1000) It is a chronic and progressive myocardial disorder, leading to sudden cardiac death in general.
- restrictive (RCM) (very rare) It implies increased stiffness and restrictive filling of the left and / or right ventricle despite a normal wall thickness.

These are hereditary cardiomyopathies. What is the link between an altered gene and the clinical phenotype? To understand this, we need to identify the molecular and functional mechanisms disrupted by mutations. Molecular and genetic defects behind these heart disorders are complex. *Drosophila* has proven to be particularly effective in discovering the molecular and cellular pathways affected in these inherited heart conditions and in identifying their genetic modifiers. We now know the regulatory cardiogenic network:
The cardiac master genes: *tinman / Nkx2-5, neuromancer / Tbx20, pannier / GATA4 / 6 and dHand / Hand*. They determine the heart during development and play a role in the functioning of the heart in adult fly and in humans.

We can present the heart of Fruitfly. It is a fairly simple linear tube located in the back. It includes two rows of contractile cells that form the myocardium. It includes the non-muscular pericardial cells aligned along the myocardial cells. *Drosophila* cardiomyocytes have a sarcomeric structure and components similar to mammalian heart cells. The heart comes from the lateral part of the mesoderm just like the heart from vertebrates. Thanks to the development of multiple heart analysis tools, we can now assess heart dysfunction in the fruitfly. The *Drosophila* electrocardiograms can now be analyzed using extracellular and intracellular electrical recordings. Optical recording of cardiac activity directly through the cuticle (based on hearts expressing the fluorescent green protein) makes it possible to observe the pulsations of the heart. The projection of infrared light through the dorsal side of the abdomen is useful for obtaining a study of periodic heartbeat inversions. A transistor-based photodiode assay and video microscopy can track heart movements. And of course there are still many other methods...

2. Fly model contributions to the understanding of channelopathies and cardiomyopathies

2.1. Channelopathies

Ion channels are proteins that cross the cell membrane and selectively control the passage of ions through it. Ion channels control cellular electrical activity and are therefore involved in every heartbeat. The ion channels are either depolarizing cells, by displacing positively charged ions in, or repolarizing cells, by displacing positively charged ions out. Mutations in the genes encoding these ion channels cause chanellopathies. More than 20 genetic diseases are linked to a dysfunction of ion channels manifested for example by disorders of the cardiac rhythmicity and the muscular contraction. Examples include the Brugada syndrome and Long QT syndrome.

Fruitfly has made great strides in studying these diseases through the identification and cloning of many genes such as the gene *Shaker*. This encodes a potassium channel *Kv* activated by voltage. The *Shaker* gene is part of the *Kv1* family of genes. *Shaker*-related genes have been identified in Fruitfly and this has enabled the discovery of other K+ channels such as *Shab, Shaw* and *Shal* and the subsequent cloning of their orthologs in mammals, *Kv2.1, Kv3* and *Kv4.3* , respectively. Mutations in the *α* subunit of the ion channels involving *KCNQ1* results in delayed repolarization. Indeed, these channels are responsible for the slow and rapid repolarization of the cardiac potassium currents. The *Drosophila KCNQ* gene shares a conserved function with its human ortholog and maintains a heart rate. Also, the older the flies get, the more their pattern of rhythmic beating deteriorates. It then presents frequent asystoles and fibrillations. These symptoms are reminiscent of those also found in aging human populations. The specific overexpression of *KCNQ* in aging flies reduces the number of

arrhythmias. This suggests that the fly heart model may be a useful alternative for studying the functions of the K+ channel in cardiac repolarization and arrhythmogenic disorders.

2.2. Cardiomyopathies

In mammals, as in Drosophila, mutations in sarcomeric or cytoskeletal/sub-membranous proteins have been implicated in the pathogenesis of hereditary cardiomyopathies.

2.2.1. HCM

Several hundred distinct mutations in more than a dozen proteins have been identified in patients with HCM. The majority of these genes code for sarcomere proteins. Data from human patients suggest that about 60% of HCMs come from dominant mutations in the sarcomere protein genes, and of these, *MYH7 and MYBPC3* predominate in frequency. As before, here are examples of discoveries made from *Drosophila* genes. For example, activation of *EGFR, Ras* and *Raf* increases the thickness of the heart wall and reduces the size of the cardiac light at diastole in adult flies.

2.2.2. DCM

DCM is caused by mutations in various genes encoding sarcomeric proteins, cytoskeletal proteins, sarcolemmal membrane and nuclear envelope proteins. These responsible genes include, for example, *dystrophin, desmin, lamin A / C*... It is not the same mutations in sarcomeric proteins that cause HCM and DCM. Here I am going to develop the very interesting example of the dystrophin gene, the effects of which can be seen in the figure 4. When the *dystrophin* gene is mutated in Fruitfly, there are several consequences: the systolic and diastolic diameters are distended, the systolic function impaired, age-related abnormalities in the cardiac myofibrillary organization. These consequences can be seen in the photos. First, there are the images of two abdominal segments of a one-week-old wildtype and of a *dystrophin* mutant heart in systole. We see thanks to the red arrowheads the distended diameters. Secondly, there is an illustration of the movements of heart tube walls over time. We can see that the diastolic and systolic diameters are more important for the *dystrophin* mutant heart.

2.2.3. RCM

RCM decreases the volumes of the two ventricles and significant diastolic dysfunction due to the decrease in the elasticity of the myocardial wall. The mutations responsible for this disease are mainly located in the genes of sarcomere proteins such as *ACTC1, TNNI3, TNNT, MYH7*. For example, expression of the hyperactive Mhc^{5} myosin in the heart tubes causes a narrowing of the heart cavity and impaired diastolic function.

3. The pathological mechanisms involved and the value of Fruitfly in modeling human cardiac diseases

3.1. The pathological mechanisms

3.1.1. Impaired calcium handling

The regulation of the contraction and relaxation of the heart is partly achieved by the cardiac signaling of calcium. There are several genes that control the amount of extracellular and

intracellular Ca^{2+}. These genes code, among other things, for the dependent Ca^{2+} voltages channels and for the Na^{+} / Ca^{2+} exchangers. A mutation in one of these genes that encode these proteins disrupts Ca^{2+} homeostasis. The consequence is a diastolic dysfunction which can cause both heart failure and heart hypertrophy. Myocardial Ca^{2+} transients can be measured using a calcium-specific fluorescent protein from the heart. According to several experiments : many genes involved in the calcium handling in cardiomyocytes are functionally conserved between *Drosophila* and mammals, like the *SERCA-interacting protein sarcolamban*.

3.1.2. Altered metabolism

The excitation-contraction coupling process of the heart is a process that requires a lot of energy. This energy is provided by Adenosine Triphosphate (ATP). However, there is a very low reserve of ATP in the heart. This is why the balance between demand and energy consumption must be controlled for proper functioning of the heart.

In Western countries, diets are often very high in fat and sugar. This increasing supply of energy to the body is responsible for an increase in triglyceride and glucose levels. This causes obesity, diabetes which are associated with heart dysfunction. The Fruitfly model is used thanks to the conservation of fat and sugar metabolisms in *Drosophila*, to study the metabolic pathways disturbed in these dysfunctions.

In addition, if Fruitfly is fed a diet high in sugar, it causes insulin resistance as well as type 2 diabetes. There is also the development of cardiomyopathies. They have a reduced lifespan, arrhythmia problems, due to the insulin pathways in particular. Like the symptoms seen in patients with type 2 diabetes mellitus, these Fruitfly have an accumulation of fibrosis-like collagen in their extracellular matrix. The main metabolic genes modulating cardiac lipotoxicity have been described using *Drosophila*. Studies describe the genetic network involving several metabolic regulators of lipotoxic cardiomyopathy including the *Insulin-TOR* pathway, and certain orthologs *ATGL, PGC1* and *SREBP*, already described for their involvement in obesity in humans.

A limited-time diet in flies shows a protective effect against age-related heart decline. 5 week flies fed a limited time of 12 hours shows improved heart function, less arrhythmia, a better fractional shortening. So a TRF diet protects against heart decline caused by a high-fat diet. And this phenomenon is mediated by the ATP-dependent TCP-1 ring complex, the mitochondrial electron transport chain complex, and the circadian clock pathways.

Cardiac aging plays a very important role in regulating metabolism. Indeed, aging flies have an alteration in cardiac metabolism with increased arrhythmia, myofibrillary disorganization and deregulation of the insulin and TOR pathways. Strangely, if we reduce the activation of insulin or the TOR pathway, we increase the lifespan and we delay cardiac aging so the aging may be controlled by nutrient-sensing.

3.1.3. Increased Oxidative Stress and Mitochondrial Dysfunction

The reactive oxygen species (ROS) are responsible for oxidative stress. For example, H2O2 and O2 are ROS. These species damage cells. Significant changes in the amount of ROS can cause heart dysfunction in Fruitfly. ROS in pericardial cells regulates the function of the heart in a paracrine way. ROS activates a kinase-dependent signaling cascade in pericardial cells and affects the functions of myocardial cells. We can explain the example of Huntington's disease. This is due to the accumulation of polyglutamine which increases oxidative stress and causes heart problems. Inducing

poly-Q repeats in cardiac tissue and an increase in density aggregates causes the increase in dihydroethidium (DHE), which reflects the production of ROS in cells. If you overexpress superoxide dismutase (SOD), an antioxidant enzyme, it cures cardiomyopathy.

The increase in reducing stress can also be toxic to the cell. The Desmin-related myopathy Drosophila model is used, using human αB-crystalline mutant flies (expressing the human mutation *CryAB*^{R120G}) which have cardiac symptoms similar to those observed in patients. This shows the involvement of the mitochondrial NADP / H metabolism in the increase in reducing stress. The human mutation *CryAB*^{R120G} expressed in flies causes an increase in diastolic and systolic diameters, a decrease in fractional shortening, as well as arrhythmias reminiscent of DCM in patients. A cardiac-specific knockdown of glucose-6-phosphate dehydrogenase, an enzyme involved in the generation of NADPH and 6-phosphogluconate dehydrogenase mitigates these heart defects.

3.1.4. Remodeling of Extracellular Matrix

Among extracellular matrix proteins, fibrillar proteins such as collagen and proteoglycans may play a role in determining the properties of the myocardium. *Drosophila* is used to perform precise genetic mapping of the candidate genes involved in heart disease. The *DSCAM* and *COL6A2* genes have been identified as the gene pair that interacts most strongly and causes a slower heart rate.

4. Challenges in CVDs Modeling

The *Drosophila* heart model has already proven itself in the study of CVDs regulatory pathways. This is made possible by:

- conservation of many factors between Fruitfly and humans (cytoarchitectural components, signaling systems carrying pathological mutations known to cause CVDs).
- fruitfly is a very simple genetic model to use so it is easy to make screens to identify mutations
- similar genetic interaction screens in vertebrates are much more difficult: take more time, are more expensive, more difficult to achieve because of genetic redundancy…
- several genetic tools have been developed in flies in recent years which have further strengthened this model and allow great investigative power compared to other model systems.

Thanks to the existence of large collections of Fruitfly mutants and existing cardiac phenotyping techniques, Fruitfly's heart is very efficient at discovering genes that affect phenotypes and reveal polygenic interactions. This work would be very difficult if not impossible to carry out in the hearts of mammals. One of the major challenges is the large number of variants of DNA sequences, generated by new sequencing technologies. This makes it difficult to interpret the results of genetic diagnostic tests for cardiomyopathies and channelopathies. Because of this complexity and to differentiate pathogenic mutations causing diseases from polymorphism, in vivo tests on the heart function of flies can be very useful.

The heart of *Drosophila* was used to easily assess the effect of pharmacotherapy. Recent studies have made it possible, for example, to test the role of pentamidine which decreases the cardiac arrhythmia and improves contractility. Drugs can also be helpful in validating the role of multiple ion channels by using specific inhibitors for those channels.

However, no model is perfect and Fruitfly has some disadvantages:

- Lack of genetic redundancy in Fruitfly therefore the associated gene regulatory networks in vertebrates may not be functional in Fruitfly
- There is an evolutionary distance and morphological differences which make it an inaccurate model.

- Because of the structure of the heart of *Drosophila,* many studies are difficult if not impossible (coronary heart disease in particular).

This seminar suggests that the molecular pathways involved in hereditary cardiomyopathies and channelopathies can be preserved between flies and humans. Indeed, many cytoarchitectural and signaling protein mutations cause similar heart defects in flies. Identifying the molecular and functional mechanisms of mutations causing heart disease is essential to understand the pathways that link an altered gene to a clinical phenotype. To conclude, *Drosophila* genetics are useful for:
- identify genes and pathways that can contribute to channelopathies and cardiomyopathies
- discover the mechanisms by which protein mutations trigger signals that cause heart reshaping
- test, verify or validate pathogenic DNA variants associated with heart disease
- screen pharmacological agents to identify new therapies.