Functional roles of somatic piRNAs and their evolution

In multicellular organisms such as animals, cells from one organ are often very different from those of another organ. For example, cells from the eye are very different than the cells of the skin. Each cell type in each organ performs a very specialized and precise function. However, despite the cells of the eye being so different from those of the skin, all the cells of an organism share the same instructions booklet, namely the same genome. This has fascinated scientists for decades; how are cells so different if they all share the same genome? What mechanisms determine differences between cells? And how have these mechanisms shaped evolution? The key to understanding the differences between cells relay on how each cell reads and interprets the genome, also known as gene expression regulation.

In recent years, scientists have discovered many different elements that contribute to regulating the gene expression at different levels, however, we are still far from having a complete and comprehensive answer to the previous questions. Among the known elements that are involved in the gene expression regulation, there are different types of small RNAs. Some of these small RNAs, such as microRNAs, have been found to have a very powerful role in regulating the expression of genes. In the 2000s another type of small RNAs was discovered in animals named piRNAs. Initially, piRNAs were believed to work as protectors of the genome, defending it from internal and external threats. However, very recently, in our previous investigations, we have observed that some of these piRNAs could also play an important and yet unknown role in regulating the gene expression. In this project, **we will explore the possible functional roles of piRNAs in regulating the gene expression, and how these roles could have impacted the animal evolution**.

In our experiments, we will use three different insect species as research organisms as representatives of three large insect orders. These are the cricket *Gryllus bimaculatus* (order: Orthoptera), the cockroach *Blattella germanica* (order: Blattodea), and the milkweed bug *Oncopeltus fasciatus* (order: Hemiptera). The choice of using insect species as research organisms is motivated by the fact that 90% of animal species described on Earth are insects, and they live virtually any terrestrial ecosystem, which makes them excellent models to study evolution. Furthermore, insects are easy to rear and work within the laboratory, and therefore they have been extensively used for genetic research for a long time. To determine the role of piRNAs in controlling the gene expression, we will perform a series of molecular biology experiments consisting of reducing the expression of key genes for the production of piRNA in three different tissues of each insect species. Subsequently, we will use high throughput sequencing technologies to quantify the levels of each piRNA and each gene in each experimental condition. **The expression reduction of piRNAs will result in a change of expression of those RNAs that are regulated by piRNAs, which will allow us to identify genes that are regulated by piRNAs.** Furthermore, by comparing the three chosen insect species, we will determine **the putative conservation of the piRNA roles across the ~395 million years that separate the three species** from their last common ancestor.

Because the role of piRNAs has not been widely explored, there are almost no computational tools to identify and analyze piRNAs. Thus, in this proposal, **we will develop a software that will allow researchers to identify and analyze piRNAs in any animal species**. The same software will be used for most of the analysis of this project.

All in all, this proposal will shed light on the roles of piRNAs in regulating the expression of genes, which will represent a step forward towards having a complete picture of how genomes are regulated to produce complex organisms.