Mechanical and genetics basis of cellularization and serosal window closure in Tribolium castaneum

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Summary

After *Drosophila melanogaster*, *Tribolium castaneum* has started to be the most used insect in evolutionary Biology. The main reason is because *T. castaneum* has a short germ development. In this kind of development just the head and the thorax are present in the blastoderm after gastrulation. Later the rest of the segments develop. This kind of development is closer to most of the insects than the kind of development of *D. melanogaster.* The development of *D. melanogaster* is long germ development which means that after gastrulation all the segments develop at the same time. Therefore, if we want to study general aspects of the development of insects, it is better to study it in *T. castaneum*. The study of the development of *Tribolium castaneum* has a lot of possible applications. Some of the applications are already in the market such as new ways of control to insect pests and some of them need more study and understanding. Therefore, it is important to study the development of *Tribolium castaneum* specially from a multidisciplinary perspective.

In this thesis we study the early development of *T. castaneum* which covers from after fertilization until serosal window closure. In mammals, after fertilization the cells start to divide, in the case of the insects the nuclei start to divide. After fertilization all the nuclei are embedded in a single membrane without separation between them. Therefore, if we are strict with the definition of a cell, shortly after fertilization, there is just one cell with a lot of nuclei called syncytium. In *T. castaneum* the nuclei divide 12 times before they separate to form cells with one nucleus. During the first nuclear divisions the nuclei are inside of the egg. After the 8th nuclear division, they migrate to the surface of the egg and form a single layer of nuclei. Once the nuclei are at the periphery of the egg, the plasma membrane surrounding the entire egg moves in between the nuclei (invagination) and separates them. Finally, after the 12th nuclear division the plasma membrane closes around the protocells, creating actual cells creating the blastoderm epithelium. This process is known as cellularization. At the blastoderm stage, the serosa starts to differentiate from the germ anlage. In most holometabolous insects, the serosa folds over the germ rudiment, forming a serosal window that finally closes so that the serosa completely covers the yolk and embryo.

In this thesis we studied the development period from a multidisciplinary perspective. In the chapter 2 we studied the mechanical interaction between cells which is important to form the cell shape during cellularization. To understand this pattern-forming process, we simulate the growth of the cells using a mechanical model comprising the nuclei, radial microtubules and actin cortex of the cells. We found that the mechanical interaction between cells lead to the formation of a Voronoi tessellation. The geometric and topological properties of the tessellations we find in our experiments quantitatively match with our simulations. Moreover, comparison with recent jamming models suggests that the tissues spontaneously organize at the highest possible density that is still on the liquid side of the jamming transition. In chapter 3, we studied the genetics of cellularization. Performing RNA sequencing, we found that the Innexin 7 gene group is central to finish the process. In Chapter 4, we made a gene screening of the genes involved in serosal window closure. We found that Laminin α1,2 produces a delay in the start of serosal window closure. As Laminin α1,2 is a subunit of the functional Laminin trimer, we also studied the other two subunits Laminin β and γ. We found that the trimer Laminin α1,2, β and γ is a key cell component in the process of serosal window closure.

In conclusion, *T. castaneum* has a lot of potential to become an important biological model. In this thesis we used this small beetle to perform a multidisciplinary study about the early development. But not just *T. castaneum* is useful to study the early development, but also it can be used in different studies with different applications, ranging from agriculture to material science.