STUDY OF THE DOWNSTREAM NETWORK OF Abd-B INVOLVED IN SPIRACLE DEVELOPMENT IN DROSOPHILA

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The development of the different morphology of the segments, in the anterior-posterior axis of the body, is controlled by the *Hox* genes. The *Hox* genes encode transcription factors expressed in partially overlapping patterns. This expression results in the activation of different downstream genes in different cells in the body that control the morphogenesis of segment specific structures. Thus, to understand how a segment specific structure is formed we need to identify genes downstream of the Hox genes and find how they control cell adhesion, division, survival etc. We already know some genes downstream of the *Hox* transcription factors, but our knowledge is superficial. With the aim of studying the control of morphogenesis I have decided to concentrate on the formation of a single organ: the posterior spiracle of the larva of *Drosophila melanogaster.*

The posterior spiracles are very conspicuous structures located on the eighth abdominal segment (A8). Because it is very easy to spot, researchers tend to describe if the mutant they are studying affects spiracle development. Because no one has specifically studied spiracle development there is a wealth of dispersed information available for study. In what follows I will briefly describe the spiracle structure, and how it develops. Finally, I will describe how I am unravelling the gene regulatory network downstream of the *Hox* gene *Abdominal-B* (*Abd-B*) in the spiracle.

The posterior spiracle is a tubular structure connecting the tracheal system to the outside (see figure). It is composed of an internal tube (the spiracular chamber) located inside a protrusion (the stigmatophore). The spiracular chamber forms a filter, the filzkörper.

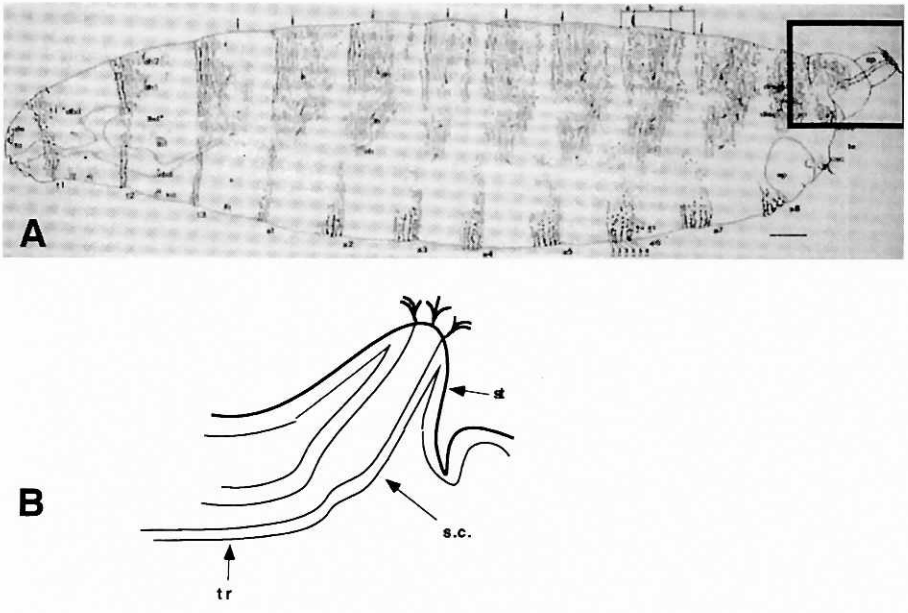

Figure 1 (A) Drawing of a first instar larva of *Drosophila* (Taken from Campos Ortega and Hartenstein). At first glance, except for the presence in A8 of the posterior spiracle (box), all segments look alike. (B) Scheme of a section of a posterior spiracle in late embryogenesis. The spiracle has already achieved its final shape. The spiracular chamber (s.c.) connects the dorsal trunk of the trachea (tr) to the spiracular opening. This structure is located in the protruding stigmatophore (st).

To find out how this simple structure is formed I am studying molecular markers that identify the cells that will form the spiracle before they are morphologically different from their neighbours. Using antibodies against the *cut* (*ct*), *empty spiracles* (*ems*) and *spalt* (*sal*) genes I have been able to describe spiracle development.

The posterior spiracle forms very rapidly. At six hours of development all the cells that will form the spiracle are still a two dimensional sheet of cells on the surface of the embryo. In two hours this sheet of cells invaginates and fuses to the primordia of the trachea. This will become the spiracular chamber. At this point the first signs of stigmatophore development can be observed as the cells that surround the spiracular chamber start protruding . The overall shape of the spiracle becoming somewhat similar to a volcano. This shape can be recognised in a 12 hour old embryo. Therefore in only six hours most of the morphogenesis of the spiracle is completed.

These morphogenetic movements are dependent on the expression of the *Abd-B* gene. In *Abd-B* mutant embryos the spiracles are not formed, and the cells in A8 behave like those in more anterior abdominal segments. Because *Abd-B* is a transcription factor this suggests that *Abd-B* is regulating downstream targets responsible for the morphogenesis of the spiracle. As a preliminary approach to identify the downstream targets, I am collecting genes that are either expressed in the posterior spiracles, or that when mutant give rise to abnormal spiracles. I am also collecting enhancer trap lines driving expression in the posterior spiracles. To assign genes to different levels in the regulatory network I am studying the expression patterns of these genes in mutants for other members of the network. In some cases due to lack of mutations in some genes the study can only be done in one direction. I will be presenting the data available to date and discuss how the network is organised. That is, to what extent the network is a simple hierarchical cascade, or to what extent there are feed-backs; redundant inputs, etc.

Finding the structure of a gene regulatory network not only has interest in itself, but also helps to pinpoint the genes that are directly regulating morphogenesis. This study will help to link pattern formation with the cellular events responsible for the actual morphogenesis.