ABSTRACT

The mushroom bodies (MBs) are uniquely indefinable brain structures present in the brains of arthropods. Functional studies have showed that the MBs are involved in higher-order behaviors such as olfactory learning and cognition. Despite wealth of knowledge on the anatomy and functions of the adult brain, little is known about their early development. Here I described the cellular and genetic mechanics that control the early development of the MBs in the *Drosophila* brain.

Using high-resolution neuroanatomical techniques, I first described the initial development of the embryonic MB primordia during embryonic stages. In the mid to late embryonic stages, the pioneer MB tracts extend along Fasciclin II (FAS II)-expressing cells and form the primordia for the peduncle and the medial lobe.

Secondly, in order to understand the cellular and genetic processes that control the development of MBs, I searched for regulatory genes expressed in the embryonic MB primordia, and found that *eyeless* (*ey*), *twin of eyeless* (*toy*) and *dachshund* (*dac*), originally implicated in eye development, are also expressed in the developing MBs. Mutations of *ey* disrupt all the neuropil structures of the MBs. A null mutation of *dac* results in marked disruption and aberrant axonal projections. Genetic analyses demonstrate that, whereas *ey* and *dac* synergistically control the structural development of the MBs, the two genes are independently regulated in the course of MB development. These data argue for a distinct combinatorial code of regulatory genes for MBs and suggest conserved roles of *Pax6* homologs in the genetic programs of the olfactory learning centers of complex brains.

Finally, based on these findings, I explored the organization of the internal substructure of *Drosophila* MBs in the course of larval development. As development

proceeds, the axonal projections of the larval MBs are organized in layers surrounding a characteristic core, which harbors bundles of actin filaments. Mosaic analyses reveals as they undergo further differentiation. Whereas the initial extensions of the embryonic MB tracts are intact, loss-of-function mutations of *fas II* cause abnormal formation of the larval lobes. Mosaic studies demonstrate that FAS II is intrinsically required for the formation of the coherent organization of the internal MB fascicles. Furthermore, I show that ectopic expression of FAS II in the developing MBs results in severe lobe defects, in which internal layers also are disrupted. These results uncover unexpected internal complexity of the larval MBs and demonstrate unique aspects of axonal extension processes during the development of the complex brain centers in the fly brain.