ABSTRACT

The aim of this thesis was to discover novel mechanisms and genetic pathways by which organs and neurons develop. This was done using the tools of the developmental geneticist to study the pharynx of the nematode *Caenorhabditis elegans*. The pharynx is a neuromuscular pump located at the anterior end of the animal and responsible for the ingestion and maceration of food. During embryogenesis, the pharynx develops from a primordium of which the cells undergo differentiation and morphogenesis. When fully developed, the pharynx can be anatomically subdivided into four portions from anterior to posterior: procorpus, metacorpus, isthmus and terminal bulb. It then consists of 62 cells of five cell types: neurons, muscle, marginal, epithelial and gland cells. This thesis had a special focus on two identical bilateral pharyngeal motor neurons, the M2 neurons that each have their cell bodies in the terminal bulb and send one axon straight through the isthmus into the metacorpus where they first turn outward laterally, then dorsally and finally towards the midline to establish a gap junction with each other.

The M2 neurons were examined in known growth cone, axon guidance and pharyngeal morphology mutant backgrounds, and this genetic analysis revealed that their proximal trajectories, i.e. between the cell body and the metacorpus, are surprisingly established through a growth cone-independent mechanism. In contrast, the distal ends of the M2 axons, i.e. within the metacorpus, are dependent on several growth cone and axon guidance pathways, such as the *unc-6* and *slt-1* pathways, for their proper development. *pha*-2 was one of the pharyngeal morphology mutants in which the M2 neurons were abnormal. We cloned *pha-2* and found it to encode a homeodomain transcription factor homologous to the vertebrate gene *Hex*. Downstream target genes for *pha-2* were also identified: *ceh-2*, the acetylcholinesterases *ace-1*, *ace-2* and *pha-2* itself. During embryogenesis, *pha*-2 is expressed in the pm5 cells that usually elongate anteriorly to form the narrow nucleus-free isthmus. In the *pha-2* mutant, the pm5 cells elongate in both directions with respect to the nucleus, and accumulate organelles within the isthmus that deform increasingly post-embryonically, possibly due to chronic contractions caused by the inability to efficiently clear excesses of acetylcholine. To identify other genes involved in the development of the pharynx, a screened was performed from which five novel mutants with defects in M2 morphology were isolated. The gene affected by one of these mutations, designated *mnm-2(et2)*, was genetically mapped then cloned. *mnm-2* encodes a zinc-finger protein related to the Krüppel-like Factor protein family. *mnm-2* is expressed within the early pharyngeal primordium, in the mother cell to M2 and its sister cell M3. Later expression is sustained only in the M3 cell, and lost in M2 soon after its birth. In the *mnm-2(et2)* mutant, *mnm-2* expression is lost prematurely in the M3 cell. The M3 cell also does not function in that mutant, as determined using an electropharyngeogram assay. Interestingly, expression of wild-type *mnm-2* in the M3 cell suffices to rescue both M3 function and the axon defects of its sister cell M2. Furthermore, killing M3 during embryogenesis phenocopies the M2 axon defects of the *mnm-2(et2)* mutant. Together the results are consistent with a model by which the anterior elongation of the pm5 cells is dependent on *pha-2*, and helps separate the sister cells M2 and M3 such that a persistent attachment of M2 to its sister cell lead to the establishment of the straight proximal trajectory of the M2 axon as M2 and M3 are pushed apart. Later, M3 depends on *mnm-2* expression for its successful differentiation, and for its ability to guide the growth cone of its sister cell M2 within the metacorpus.