Identification of genes involved in the regulation of border cell migration of the fruitfly *Drosophila melanogaster*.

Ph.D. thesis

Summary

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Background

Cell motility is an important aspect of the development of multicellular organisms. Many cell populations and tissues are known to be derived from precursor cells which were differentiated at places far from the final location. These precursor cells sometimes have to perform a long migration during which they follow a path to find their target place. For example primordial germ cells in *Drosophila melanogaster* are determined during early emryogenesis and migrate from the posterior pole of the embryo towards the presumptive gonad. Neural crest cells originate at the site of neural tube closure of the vertebrate embryo and later migrate along specific pathways in the developing animal to give rise to a large range of different tissues, including neurones, melanocytes, cartilage and bones in the head. Myoblasts also spread through the embryonic body and form musculature of the limbs.

Less frequently cells also migrate in the adult organisms. During immune response leukocytes penetrate through the blood vessels and migrate to the sites of infection. After that they have to find their way to the lymphatic organs. During wound healing when fibroblasts move to the place of injury where they start to proliferate and synthesise components necessary for wound closure.

Many pathologic conditions are caused by the improper regulation of cell migration. Tumour cells can undergo malignant transformation, detach from the tumour, and colonise other organs. Such metastatic changes increase the difficulties of medical treatment.

The above examples demonstrate the importance of understanding how migratory cells are determined, what are the regulatory processes that make them able to migrate, and what are the molecular components of the guidance system which help the cells to find their target.

Modular misexpression screen

To address these questions *in vivo* we studied the migration of border cells in the fruitfly *Drosophila melanogaster*. Border cells are a group of 6-8 specialised follicle cells which perform a stereotypic migration during oogenesis. At the beginning of stage 9 of oogenesis they delaminate from the epithelial monolayer surrounding the germline cluster, then migrate through the nurse cells towards the posteriorly located oocyte. This migration is known to be guided by two redundantly acting guidance system, based on the function of two receptor tyrosine kinases, EGFR and PVR. Although the basic nature of the chemotactic guidance has been described, the signalling events downstream of these receptors are hardly understood.

In order to identify molecular components of the migratory system we performed a large scale modular misexpression screen. In *slbo* mutant background 1510 randomly targeted genes of the third chromosome were overexpressed in border cells and these

flies were scored for the suppression of a defective migration phenotype. The genes behaving as suppressors in that screen are expected to code for rate-limiting factors for cell migration in this situation or are positive regulators of migration and when overexpressed are able to force cells to get migratory.

In this screen we found 72 positive lines. Out of them 59 lines were further characterised. 50 EP transposon insertions were found to be inserted near to genes. These genes encoded proteins functional in a wide scale of cellular processes. For instance we found inserts near to genes encoding actin binding proteins, transcription factors, protein kinases or house keeping proteins.

Actin rearrangements are fundamental in migrating cells. The major force needed for the movement is supported by the network of actin filaments. The polymerisation of actin at the leading edge of moving cells pushes the membrane forward and the extending protrusions (lamellopodia, filopodia) establish stabile contact with the substrate.

Without this cellular process cells are not able to move. We wanted to investigate how actin rearrangements are regulated in migrating border cells. For that reason we chose for further analysis the EP lines that had inserts at genes likely involved in the regulation of actin cytoskeleton rearrangements.

Cortactin

One of the genes identified in the screen was *cortactin*. Cortactin protein is a Src substrate that interacts with F-actin and can stimulate actin polymerisation by directly interacting with the Arp2/3 complex. Generating imprecise excision of the EP insert 622 base pairs upstream of the gene we have isolated the first loss-of-function mutants of the single *Drosophila cortactin* gene. Mutants were viable and fertile, showing that *cortactin* is not an essential gene.

However, *cortactin* mutants show distinct defects during oogenesis.

During oogenesis, Cortactin protein is enriched at the F-actin rich ring canals in the germ line, and in migrating border cells. In *cortactin* mutants, the ring canals are smaller than normal. A similar ring canal phenotype has been observed in *Src64* mutants and in mutants for genes encoding Arp2/3 complex components, supporting that these protein products act together in vivo. *Cortactin* mutants also show impaired border cell migration. We find that the accumulation of Cortactin protein is positively regulated by PVR, one of the guidance receptors in border cell migration. Also, overexpression of Cortactin can by itself induce F-actin accumulation and ectopic filopodia formation in epithelial cells.

We performed epistasis experiments which demonstrate that Cortactin is one of the factors acting downstream of PVR and Src to stimulate F-actin accumulation. Cortactin is only a minor contributor in this regulation, consistent with the *cortactin* gene not being essential for development.

Myoblast city and Sponge

Another gene identified in the genetic screen was *Myoblst city*. *Myoblast city* (*mbc*) is homologous to mammalian DOCK180, a potent activator of Rac1. Rac1 activation is known to lead to multiple changes in the actin cytoskeleton network. Using null mutant of *mbc* in mosaic analysis we found that in the absence of MBC the border cells migrate very poorly. This phenotype is similar to the one observed in *Rac1* loss-of-function situation. Performing epistasis experiment we found evidence that MBC is involved in the downstream signalling pathway of PVR and probably through Rac1 MBC mediates the effects of the guidance receptor to the actin cytoskeleton.

We also analysed the closest *Drosophila mbc*-homolog, *sponge*. *sponge* mutation alone did not influence the migration but strongly enhanced the border cell delay caused by *mbc* loss-of-function. Sponge also enhanced the suppression of the effect of activated PVR.

These data suggest an important role of *myoblast city* and *sponge* and high redundancy between these two related genes acting in the migratory process downstream of PVR.

Mal-d

Based on homozygous bristle phenotype similar to the ones of certain actin regulating genes we identified a new gene potentially involved in actin cytoskeleton reorganisation. The gene named *mal-d* is the *Drosophila* homolog of mammalian genes encoding for MAL/Myocardin proteins, transcriptional cofactors of Serum Response Factor (SRF).

The mutants of *mal-d* generated by EMS mutagenesis and imprecise excision of an EP element showed different phenotypes, each of them related to defects of actin polymerisation. The actin cytoskeleton of the mutant follicle cells was visibly reduced. Similar but more pronounced effect was observed in border cells which also had strong migration delays. By analysing the loss-of-function effects of

mal-d and *SRF* mutants we found that they significantly overlap. The overexpression of the activated form of MAL-D caused the opposite effect, a robust accumulation of polymerised actin. Non of these

loss-of-function or gain-of-functions effects caused changes of the amount of monomeric actin in cytoplasm.

These observations suggest that mal-d may positively regulate transcription of genes encoding proteins involved in the building up and/or maintenance of actin networks.