Appendix

Summary, samenvatting, curriculum vitae and stellingen
Summary

mRNA transport and targeting are essential to gene expression regulation. Specific mRNA sequences can bind several proteins and together form RiboNucleoProtein particles (RNP). The various proteins within the RNP determine mRNA fate: translation, transport or decay. RNP composition varies with localization, cell cycle and environmental cues. RNPs can move freely throughout the cytoplasm or bind subcellular cell structures depending on the contained mRNA. RNPs are associated with ribosomes when the mRNAs need to be translated, with transport granules if the mRNAs are targeted to a specific cellular location or with Processing Bodies (PB) or Stress Granules (SG) if translation needs to be turned down or the mRNAs are to be degraded. Insight into the mechanisms dictating RNP transition from one state to the other will lead to improved understanding of gene regulation itself. Therefore, this thesis aimed at investigating mRNA dynamics. Most experiments were performed in Drosophila embryonic muscles as they can be visualized in the whole living organism placed on a microscope stage, thus approaching endogenous conditions.

Besides, several strategies involving gene or cell therapies to treat myopathies are being developed, implying that therapeutic mRNAs have the ability to move throughout the entire myoplasm. This was another reason for using muscle cells in most of our experiments.

Direct observation of mRNA movement in Chapter 2 reveals that mRNA does not move through the entire cell but is restricted to several subdomains. Rapid motion within one domain is observed but exchange between the domains is limited. These boundaries of yet undetermined nature might restrict mRNA translation, and stand in the way of a few above mentioned cell therapies. Chapter 3 further outlines the presence of these domains using a more objective quantitative image analysis tool. Chapter 4 shows that the domain boundaries are resistant to hypoxia. Low oxygen concentrations induce the formation of mRNA containing SG in the cytoplasm of embryonic muscle cells. mRNAs can move in and out of these granules but still no exchange is observed between the muscle domains. Chapter 5 reveals that two mRNA binding proteins, PUM-GFP and GFP-
dFMR1, which are present in similar granule types, have different dynamics. Although GFP-dFMR1 contains a fast moving fraction, it shuttles faster in and out of the granules than PUM-GFP. Finally Chapter 6 gives an overview of a few parameters which are essential to quantitative image analysis. Indeed, thorough control of each image acquisition parameter appeared essential to a relevant interpretation of the obtained results.

This work exposes a few new aspects of mRNA dynamics in a living Drosophila model. Extended use of this system to unravel the nature and functioning of the muscle domains will lead to improved knowledge on translation regulation.