Abstract

Every higher organism consists of several hundreds of different cell types. The cell differentiation is a process which requires highly precise regulation of mRNA production and subsequently protein synthesis. During development of an organism each cell gains a unique palette of mRNA and protein molecules, which reflects the cell´s fate. To understand the basic function and regulation of genes that are important during development, mRNA expression profiling is an irreplaceable tool. However, most techniques to determine the mRNA content, such as Northern blot, microarrays and *in situ* hybridization have some limitations in their specificity, dynamic range and/or sensitivity. Quantitative real-time PCR analysis (qPCR) for nucleic acids quantification was introduced in the last decade and it has overcome the above mentioned drawbacks. qPCR has rapidly become the golden standard in basic research as well as in many aspects of applied research such as molecular diagnostic, food pathogen detection and genetically modified organism (GMO) analysis.

We decided to apply qPCR in studies where it helps us to understand basic biological processes that take place in the developing organism. One of the focus areas of the Laboratory of gene expression at IMG AS CR, where I did my PhD thesis, was the role of Src tyrosine kinases in the early development of vertebrates studied on the African clawed frog *Xenopus laevis*. *Xenopus* oocyte and early embryos are huge compared to mammalian ones and contain enormous amount of biological material (RNA, proteins, ribosomes and mitochondria), which can be otherwise obtained only from thousands of somatic cells. Therefore, *Xenopus* has become one of the most popular model organisms for developmental studies.

I was dealing with five projects during my PhD studies:

1. To find reference genes suitable for normalization of temporal analysis of mRNA expression throughout early developmental period of *Xenopus*. Our qPCR examinations revealed that no one of so far routinely used *Xenopus* reference genes met criteria for a developmental reference gene. Instead, we found out that reliable data can be obtained by normalization against total RNA content in individual samples.

2. To determine time profiles of expression of a group of developmental genes at early development of *X. laevis*. To compare these profiles with predicted function(s) of these genes. The expression profiles of 21 important genes during early development were determined and tight connection between the roles of the genes in different developmental stages and their expression was found. New methods for data pretreatment and statistical analysis of multidimensional data were tested on our results.

3. To determine temporal and spatial expression profiles of *Xenopus* Src tyrosine kinases (STK) and Csk , the natural inhibitor of STK at early development. This was achieved by qPCR analysis and whole mount *in situ* hybridization analysis.

4. To determine profiles of spatial distribution of developmental mRNAs within *X. laevis* oocytes. For this purpose a new method, qPCR tomography was developed. mRNA molecules were found to form two distinct gradients along the animal – vegetal axis of the oocyte. The first group is predominantly localized in the animal hemisphere and consists of mRNA expressed from genes such as FoxH1, Oct60, Xmam, elongation factor 1-alpha, GAPDH, GSK3-beta, disheveled, beta-catenin, Tcf-3 and Xpar1. The second group of mRNAs forms a gradient with a maximum in the vegetal hemisphere and consists of mRNAs expressed from genes such as VegT, Vg1, Wnt11, Otx1, Deadsouth, Xcad2, Xpat and Xdazl.

5. To analyze gene expression during the immune response in flash-fly *Sarcophaga bullata*. The expression profiles of 8 genes (transferrin, sapecin, Ppo1, Ppo2, storage binding protein, cathepsin L, 18S rRNA, sarcocystatin) predicted to be involved in immune response were determined in collaboration with the Institute of Organic Chemistry and Biochemistry, AS CR in Prague.

Our results indicate that qPCR is a suitable method for studies of gene expression and mRNA localization during early development. Results obtained during my PhD studies were presented in 7 papers, in several oral presentations at international conferences and in numerous posters. One manuscript is in preparation.