Study of the structure and function of the *Drosophila melanogaster* Fcp1 phosphatase

SUMMARY OF PHD THESIS

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Introduction

The enzyme responsible for the transcription of protein-encoding genes in eukaryotic organisms is RNA Polymerase II (RNAP II). Its largest subunit (Rpb1) contains a unique domain on its C-terminus, consisting of tandem repeats of the Tyr-Ser-Pro-Thr-Ser-Pro-Ser consensus sequence. The number of repeats varies among organisms (26 in yeast, 52 in human), but the sequence is conserved. This C-terminal domain (CTD) is essential for cell viability. Many studies proved that the CTD can be highly phosphorylated, and the level of phosphorylation is not constant. RNAP II has two well defined conformational states: RNAP IIO (hyperphosphorylated) and RNAP IIA (hypophosphorylated), which have different functions during transcription. In IIA form the enzyme is able to bind into the preinitiation complex that assembles on the promoter. Phosphorylation of the CTD happens during initiation, and allows promoter clearance, thus elongation is done by the IIO form. The CTD has to be dephosphorylated in order for the enzyme to be able to start another round of transcription. In addition, the CTD is a binding site for a number of proteins that interact with the RNAP II, whose binding is influenced by the pattern of phosphorylation on the CTD. This means, that CTD kinases and phosphatases have a cardinal role in gene expression regulation. Many CTD modifying kinases and a few phosphatases have been identified, from the latter group the most well known is the TFIIF interacting CTD phosphatase, Fcp1.

Fcp1 has mainly been studied with *in vivo* methods in yeast, from higher eukaryotes mostly only *in vitro* results are available. During my research I created a system, with which I can get *in vivo* information about the role of Fcp1 in a higher organism. As model I chose the *Drosophila melanogaster*, because of the large number of genetic and biochemical methods available to study it.

Aims

The aims of my study were the analysis of the function of Fcp1 and the investigation of its role in transcription. The central role of Fcp1 in the dephosphorylation of the CTD is well known, however, the precise mechanism and the contribution of Fcp1 to the regulation of different sub-processes (like capping, splicing and polyadenilation) is not clear yet. Without solid knowledge of these processes we cannot get a complete picture of transcription, and gene expression regulation. I set the *Drosophila melanogaster* orthologue of Fcp1 as the focus of my studies. *Drosophila* is a great model organism, easy to handle, its genetics is well established, and as a higher eukaryote, the conclusions based on *Drosophila* experiments can be easier applied to e.g. human results.

Since there was no data on the *Drosophila* orthologue of Fcp1, my first aim was to identify and clone the gene of dmfcp1.

Fcp1 is found in every eukaryote, its function is conserved, so I found it interesting to study if a given orthologue is able to influence transcription in a heterologue system, e.g. DmFcp1 in human cells.

I is not clear yet, if the interaction between Fcp1 and Rpb4 is significant to the function of Fcp1. Thus another aim was to study the interaction between *Drosophila* Fcp1 and Rpb4, and to identify the regions required for this interaction.

To investigate the function of Fcp1, I wanted to change the in vivo level of Fcp1 with transgenes that are able to overexpress or silence the dmfcp1 gene.

Methods and Materials

In vitro recombinant DNA techniques
Nucleic acid preparation
Polymerase chain reaction, reverse transcription-coupled PCR
Yeast two-hybrid experiments
Establishment of transgenic *Drosophila melanogaster* lines with P-element insertion
In vivo functional studies with the UAS/GAL4 system
Drosophila wing disc staining with acridine-orange
Handling and transformation of human (HeLa) cell culture
Handling and transformation of *Drosophila* S2 cell culture
Measurement of luciferase activity

Results

Since there was no data on the *D. melanogaster* orthologue of Fcp11, it had to be identified first. I identified the gene encoding dFcp1 (CG12252) with the BLAST search engine, then cloned the cDNA and genomic sequence.

According to the literature, yeast and human Fcp1 interact with Rbp4, the fourth largest subunit of RNAP II. I showed with yeast two hybrid experiments, that the product of the gene which I identified – CG12252 – is also able to bind to DmRPB4. Furthermore I found that DmFcp1 contains a transcription activation domain on its C-terminus. This also coincides with other studies of Fcp1.

To influence the level of Fcp1 *in vivo*, I created transgenic *Drosophila* lines; the transgenes can be expressed with the UAS/GAL4 system. This system makes it

possible to express a given sequence with spatial and/or temporal specificity. In *Drosophila melanogaster* both the overexpression and the silencing of the dmfcp1 gene causes lethality or the animals develop different aberrations on certain organs, depending on the type of GAL4 driver used. The observed phenotypes suggest that apoptosis occurs in the affected animals. I showed with acridine-orange staining that this is indeed the case. In those tissues, where the level of Fcp1 has been changed, a large number of cells can be seen in apoptosis.

A key protein in apoptosis is p53, so we raised the question, whether p53 has a role in the phenotypes caused by the change in Fcp1 level. We showed that in the absence of p53 the phenotypes of decreased Fcp1 level are less severe, while if we overexpress a dominant-negative form of p53 (one which is unable to activate transcription) the phenotypes get more severe. This dominant-negative p53 cannot activate the transcription of its target genes that are components of the apoptotic cascade, but it can take part in the induction of apoptosis in a transcription-independent manner. In this case this is understandable, in light of the assumption that the change in Fcp1 level would block transcription in general, making a transcription-dependent pathway ineffective.

The conserved function of Fcp1 raised the question, whether a given orthologue would be able to have an effect in another organism. Cell culture experiments indicate that overexpression of DmFcp1 can inhibit transcription of HeLa cells, and the R251G point mutant (in the phosphatase domain) decreases the transcription activity even in smaller quantities. This might be caused by a competition of endogenous and ectopic proteins.

Further analysis of the promoter of dmfcp1 can shed light on the different factors that regulate the level of Fcp1 during development and in different tissues.

My results show that the correct function of Fcp1 is essential for the normal development of *D. melanogaster*. Its level is tightly regulated, since both the overexpression and the silencing of its gene leads to the death of the animals or to the

development of aberrant tissues/organs. These defects are caused by the blockage of normal transcriptional events, in which case the cell undergo apoptosis. The tumor suppressor p53 plays an important role in this, without activating its target genes, by a transcription-independent pathway. I created a system, in which the function of Fcp1 and the role of CTD phosphorylation can be investigated *in vivo* in a higher eukaryote.

List of publications

Publications directly used in and related to the thesis:

The RNA Pol II CTD phosphatase Fcp1 is essential for normal development in Drosophila melanogaster.
Tombácz I, Schauer T, Juhász I, Komonyi O, Boros I.
Gene. 2009 Oct 15;446(2):58-67. Epub 2009 Jul 24.

Misregulated RNA Pol II C-terminal domain phosphorylation results in apoptosis.
Schauer T, Tombácz I, Ciurciu A, Komonyi O, Boros IM.
Cell Mol Life Sci. 2009 Mar;66(5):909-18.

Other publications:
GAL4 induces transcriptionally active puff in the absence of dSAGA- and ATAC-specific chromatin acetylation in the Drosophila melanogaster polytene chromosome.
Ciurciu A, Tombácz I, Popescu C, Boros I.
Chromosoma. 2009 Aug;118(4):513-26. Epub 2009 May 2.

Conference presentations and posters

30th FEBS Congress – 9th IUBMB Conference, 2005

Structure and function of the *Drosophila melanogaster* Fcp1 phosphatase (poster)

I. Tombácz, I. Török and I. M. Boros

20th International IUBMB Congress, 2006

The role of the *Drosophila* melanogaster FCP1 phosphatase in transcription regulation (poster)

I. Tombácz, A. Ciurciu, O. Komonyi and I. M. Boros

Workshops of Genetics in Hungary – VI. Miniconference, 2007

The role of the *Drosophila* melanogaster FCP1 phosphatase in transcription regulation (presentation)

Meeting of the Hungarian Biochemistry Association, 2008

The role of the RNA polymerase CTD phosphatase Fcp1 (presentation)

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