COORDIGENE

BIOLOGIE & SANTÉ 2011

Coordination of gene expression from transcription to translation

Programme blanc 2007

Coordinateur: D. Auboeuf; Partenaire: S. Vagner

Scientific context and aims

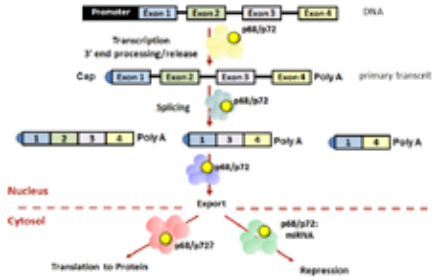
Gene expression is a multiple step process including transcription, capping, splicing, RNA 3'-end processing, mRNA export and translation. Increasing evidences indicate that all these steps are tightly connected, but the molecular actors of the coupling between gene expression steps have not been fully characterized yet. In this project, we focused on two highly-related members of the DEAD box family of RNA helicases, p68 (ddx5) and p72 (ddx17), that have been shown to play a role in different gene expression steps. Our aims were to:

1. Develop tools to identify genes regulated at the transcriptional and post-transcriptional level by p68 and p72 in different cellular contexts.
2. Characterize the cellular programms depending on p68 and p72 in different cellular models.
3. Identify the molecular mechanisms by which p68 and p72 control gene expression at the transcriptional and post-transcriptional level.

Results

P68/p72 and transcription

P68/p72 have been reported to act as transcriptional coregulators of key transcription factors like the estrogen receptor. We performed Exons Arrays after estradiol treatment of breast cancer cells after depletion or not of p68 and/or p72. We found that estradiol differentially regulates protein isoforms produced from the same genes. In particular estradiol increases a NET1 isoform favouring cell proliferation and decreases the NET1 isoform favouring cell adhesion. Remarkably, p68 and p72 are required for this switch that participates in the estradiol effects on cell proliferation (Dutertre et al. Cancer Research 2010a). Furthermore, a third of the genes activated by estradiol require p68 and p72. As depletion of p68 and p72 impairs Pol II recruitment of estradiol-activated promoters and impairs pre-mRNA production in response to estradiol, our data are consistent with a role of p68 and p72 as an estrogen receptor co-activator. However, a third of the genes repressed by estradiol treatment require also p68 and p72. In this case we did not see any effect on pol II recruitment and pre-mRNA production. In this context, we recently showed that estradiol treatment affects miRNA processing (Maillot et al., Cancer Research 2010) and a recent report suggests that these effects are mediated by p68/p72. Therefore, our hypothesis is that p68 and p72 control estradiol-mediated gene transcriptional activation as transcriptional co-activators and estradiol-mediated mRNA degradation through miRNA processing. These data highlight the requirement of integrating different layers of regulation to better understand signalling pathways. Furthermore, targeting the activity of p68/p72 in either transcription or miRNA processing may allow to control different sets of estradiol-regulated genes.



P68/p72 and splicing

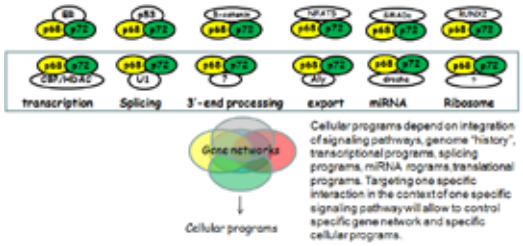
During the time course of this project, we improved and validated tools for the analysis of the transcriptome at the exon level by using Affymetrix Exon Arrays (De la grange et al. Nucleic Acids Research, 2010). Using these tools, we identified splicing variants associated with metastases (Dutertre et al. Cancer Research 2010b). Remarkably, p72 is over-expressed in breast cancer tumors giving rise to metastases when compared to breast tumors that do not give rise to metastases. This result is in agreement with recent reports indicating mis-regulation of p72 and/or p68 in several cancer models. A large set of alternative spliced exons (corresponding to more than 300 genes) depending on p68 and/or p72 were identified. About two thirds of these exons require p68 and/or p72 to be included. One characteristic of these exons is the high GC content in the intron/exon boundaries which correlates with secondary structure stabilisation. Furthermore, p68/p72 depletion impacts on the recruitment of some spliceosome components on these exons. In terms of functional impact, p68/p72 control the splicing of the macrohistone H2AFY that play a role in large chromosome domain structure and that has been involved in cancer. Our hypothesis is that the splicing activity of p68/p72 impact on splicing of genes involved in tumor progression opening up the possibility of targeting this function to impact on cell behaviors.

P68/p72 and mRNA fate

Using Exon Arrays, we oobserved that about 170 alternative last exons (in addition to internal exons) were differentially affected by p68/p72 depletion. Working on selected cases, we are currently testing whether p68 and p72 are involved in transcription termination and/or RNA 3'-end processing as recently suggested. However, we are also analysing the impact of p68/p72 on miRNA levels as several recent reports indicate that p68/p72 are involved in miRNA processing. We anticipate that the differential effects of p68/p72 on the alternative exons could also be due to differential stability of transcripts mediated by miRNAs.

Furthermore, while analyzing the impact of p68 on the estradiol-stimulated c-fos gene, we observed that p68 depletion resulted in the increase in the nuclear level of the c-fos mRNA while decreasing its cytosolic level. Interestingly, p68 co-immunoprecipitated specifically the c-fos mRNA and interacted with Aly involved in mRNA export. P68 is in fact a bona fide mRNA export factor as it shuttles between the nucleus and the cytosol.

More recently, we observed that p68 controls the cytosolic fate of a large population of mRNAs. Indeed, micro-array analysis of transcript distribution between polysome and non-polysome fractions demonstrated that p68/p72 depletion resulted in a shift of about 3,000 transcripts from polysome to non-polysome fractions, suggesting a role of p68/p72 in control of the cytosolic fate of a large population of mRNAs. In this context, p68 and p72 have been shown to impact on rRNA processing and interact with fibrillarin which is involved in rRNA processing and methylation. The effect of p68/p72 on mRNA distribution within ribosomes could be either a general effect on ribosomes or on selective effects on a subset of transcripts.



Conclusions and Future directions

During the time course of this work, we developed tools allowing to identify at a large scale level genes regulated by p68 and p72 at the transcriptional and post-transcriptional level in different cellular models. Our data demonstrate that these RNA helicases play a key role in several steps of the gene expression process. We demonstrate that p68 and p72 control key cellular programms that play a role in cancer progression. Finaly, the characterization of the molecular mechanisms by which p68 and p72 control gene expression at the transcriptional and post-transcriptional level will allow to set up new pharmacological strategies. In this context, several p68 and p72 mutants have been cloned in order to determine the domains critical for the involvement of the proteins in different steps of the gene expression process. Such mutants will also be necessary to demonstrate the direct involvement of p68/p72 in different layers of the gene expression process. We will also use an aptamere reported to block p68 helicase activity to test its impact on various cellular programs and signalling pathways and on cell behaviour.

Publications

1. Cotranscriptional exon skipping in the genotoxic stress response. Dutertre M, Sanchez G, De Cian MC, Barbier J, Dardenne E, Gratadou L, Dujardin G, Le Jossic-Corcos C, Corcos L, Auboeuf D. Nat Struct Mol Biol. 2010 Nov;17(11): 1358-66.
2. Alternative splicing and breast cancer. Dutertre M, Vagner S, Auboeuf D. RNA Biol. 2010 Nov 19;7(4):403-11.
3. Estrogen regulation and physiopathologic significance of alternative promoters in breast cancer. Dutertre M, Gratadou L, Dardenne E, Germann S, Samaan S, Lidereau R, Driouch K, de la Grange P, Auboeuf D. Cancer Res. 2010 May 1;70(9): 3760-70.
4. Splicing factor and exon profiling across human tissues. de la Grange P, Gratadou L, Delord M, Dutertre M, Auboeuf D. Nucleic Acids Res. 2010 May;38(9):2825-38.
5. Exon-based clustering of murine breast tumor transcriptomes reveals alternative exons whose expression is associated with metastasis. Dutertre M, Lacroix-Triki M, Driouch K, de la Grange P, Gratadou L, Beck S, Millevoi S, Tazi J, Lidereau R, Vagner S, Auboeuf D. Cancer Res. 2010 Feb 1;70(3):896-;
6. Widespread estrogen-dependent repression of micrornas involved in breast tumor cell growth. Maillot G, Lacroix-Triki M, Pierredon S, Gratadou L, Schmidt S, Bénès V, Roché H, Dalenc F, Auboeuf D, Millevoi S, Vagner S. Cancer Res. 2009 Nov 1;69(21):8332-40.

CONTACT :

Didier.auboeuf@inserm.fr