

RNAQuality Meeting

Granada, Spain, 11-13 June 2008

Final Programme

Wednesday 11th of June 2008

- 18:30 Arrival and registration
Buffet dinner
- 19:40-20:00 Welcome and Introductory remarks
Michael Kiebler and **Astrid Lunkes**

Session 1: QC of nascent RNA PolII transcripts Part I

Chair: **Elisa Izaurralde**

- 20:00-20:25 **Dmitry Belostotsky** (University of Missouri, US)
*Genome-wide high-resolution mapping of the plant
exosome substrates*
- 20:25-20:50 **Thomas Gingeras** (Affymetrix, Inc., US)
*New Classes of Functional Short RNAs and Chromosome-
wide Transcriptional Networks*
- 20:50-21:15 **Alain Jacquier** (Institut Pasteur, Paris, FR)
*The complete map of CUTs in *Saccharomyces
cerevisiae*: implications for CUTs metabolism and
functions.*
- 21:15-21:40 **Torben Heick Jensen** (University of Aarhus, DK)
*Robust transcription activity immediately upstream
the majority of active mammalian promoters*
- 21:40 **Welcome drinks**

Thursday 12th of June 2008

Session 1: QC of nascent RNA PolII transcripts Part II

Chair: **Hervé Le Hir**

- 08:45-09:10 **Steve Buratowski** (Harvard University, US)
Exosome and the Nrd1/Sen1 Complex
- 09:10-09:35 **Domenico Libri** (CNRS, CGM, Gif-sur-Yvette, FR)
From transcriptional noise to the sound of regulation
- 09:35-10:00 **Steve West** (University of Oxford, UK)
Transcriptional termination of RNA polymerase II enhances gene expression
- Ana Rondon** (University of Oxford, UK)
Failsafe mechanisms in Pol II termination
- 10:00-10:25 **Neus Visa** (Stockholm University, SE)
Cancelled
- 10:00-10:25 **Coffee break**

Session 2: mRNA QC pathways Part I

Chair: **David Tollervey**

- 10:45-11:05 **Allan Jacobson** (University of Massachusetts, US)
Nonsense-mediated mRNA decay in yeast
- 11:05-11:25 **Hervé Le Hir** (CNRS, CGM, Gif-sur-Yvette, FR)
Reconstitution of the human NMD surveillance complex
- 11:25-11:45 **Andreas Kulozik** (University of Heidelberg, DE)
Alternative pathways in mammalian NMD
- 11:45-12:05 **Stuart Peltz** (UMDNJ, US).
Cancelled (replaced by **Allan Jacobson**)
- 12:05-12:25 **Andrzej Dziembowski** (Warsaw University, PL)
Novel endonuclease activity of the exosome contributes to digestion of its physiological substrates.
- 12:30-13:30 **Lunch**
- 13:30-15:00 **Poster presentation**

Session 2: mRNA QC pathways Part II

Chair: **Torben Heick Jensen**

- 15:00-15:25 **Elisa Izaurrealde** (MPI Tübingen, DE).
Cancelled (replaced by **Hélène Gaillard**, Uni Sevilla, ES)
- 15:25-15:50 **Oliver Mühlemann** (University of Bern, CH)
SMG6 induced endonucleolytic cleavage of nonsense mRNA in human cells
- 15:50-16:00 **Mariangela Morlando** (University of Rome, IT)
Short talk: *Drosha processing and microRNA transcription*
- 16:00-16:10 **Gadi Schuster** (Technion, IL)
Short talk: *The evolution of RNA Polyadenylation; Different Tails Tell Different Tales*
- 16:10-16:30 **Coffee break**

Session 3: Structural organization of QC players

Chair: **Nick Proudfoot**

- 16:55-17:20 **Haiwei Song** (Inst. Molecular & Cell Biology, SG).
Cancelled (replaced by **Nicholas Proudfoot**, Uni Oxford, UK)
- 17:20-17:45 **Ailong Ke** (Cornell University, US)
Architecture of the yeast exosome complex suggests routes of RNA recruitment for 3' processing
- 17:45-18:10 **Marc Graille** (University Paris-Sud, FR)
Structural studies of proteins involved in mRNA quality control pathways
- 18:10-18:35 **Esben Lorentzen** (Birkbeck College, London, UK)
Crystal structure of the active subunit of the yeast exosome core, Rrp44, bound to RNA
- 18:35-18:45 **Laura Milligan** (University of Edinburgh, UK)
short talk: *A new yeast exosome cofactor Mpp6 and redundant activities contribute to the rapid degradation of ncRNA transcripts*
- 19:00-20:00 **Dinner**
- 20:00-21:30 **poster presentation and discussion**

Friday 13th of June 2008

Session 4: Localization and translation Part I

Chair: **Michael Kiebler**

- 09:10-09:35 **Ilan Davis** (University of Oxford, UK)
The Mechanism of mRNA Localisation and Anchoring in Drosophila
- 09:35-10:00 **Stefan Hüttelmaier** (University of Halle-Wittenberg, DE)
ZBP1, the postman controlling spatially restricted β -actin translation in primary neurons?
- 10:00-10:25 **Rob Singer** (Albert Einstein College of Medicine, NY, US)
Following the mRNA regulation and quality control in living cells
- 10:25-10:50 **Sadanand Vodala** (Brandeis Uni, US)
Cancelled
- 10:25-10:50 **Coffee break**

Session 4: Localization and translation Part II

Chair: **Bertrand Séraphin**

- 11:10-11:35 **Eric Phizicky** (University of Rochester, US)
*Degradation of several hypomodified mature tRNA species in *Saccharomyces cerevisiae* is mediated by Met22 and the 5'-3' exonucleases Rat1 and Xrn1*
- 11:35-12:00 **Joel Richter** (University of Massachusetts, US)
Translational Control of Cellular Senescence
- 12:00-12:25 **James Anderson** (Marquette University, US)
TRAMP mediated degradation of RNA in yeast
- 12:25-12:50 **David Tollervey** (University of Edinburgh, UK)
Getting Systematic with Ribosome Synthesis
- 12:50-13:15 **Michael Kiebler** (University of Vienna, AT)
mRNA transport, mRNA decay and miRNA silencing in neurons
- 13:30-14:30 **Lunch and departure**

International conference “RNA Quality” in Granada, Spain, from June 11-13 2008

Scientific Report

The idea to organize this conference was born at an ESF kick-off meeting in Strasbourg, France that took place on the 11th of May 2007. Here, amongst others, the three Project Coordinators from the three funded RNAQuality Collaborative Research Programmes (CRPs), Bertrand Séraphin, Torben Heick Jensen and Michael Kiebler, met to discuss future networking activities. We all agreed to organize a conference bridging the diverse topics of the three teams: RNA quality control with a special emphasis on the exosome on one side and RNA localization and translational control on the other.

As already mentioned, the conference brought together participants of three different CRPs of the RNAQuality Programme. In addition, 15 external experts on the different topics were invited to talk. The symposium was attended by 52 people ranging from young students and postdocs to international experts on the various topics. From the view of the organizers, the conference was thought to serve several important goals. Firstly, to initiate collaborations across individual CRPs within the RNAQuality Programme. Secondly, to have a status report on both RNA quality and RNA localization; where are the respective fields and what are the urgent open questions?

The following four major sessions took place:

1. Quality control of nascent RNA Polymerase II transcripts
2. mRNA quality control pathways
3. Structural organization of quality control players
4. RNA Localization and translation

Quality control of nascent RNA Polymerase II transcripts

The first session was chaired by Michael Kiebler (first half) and by Hervé Le Hir (second half). In total, nine speakers focused on mechanisms of transcription termination of RNA polymerase II (RNAPII) and the fate of the produced transcripts. In the first half, four talks discussed the pervasive transcription occurring in genomes of plant (Dmitry Belostotsky, University of Missouri, Kansas City, USA), yeast (Alain Jacquier, Institut Pasteur, Paris, France) and human (Thomas Gingeras, Cold Spring Harbor Laboratory, New York, USA and Torben Heick Jensen, Aarhus University, Denmark). Tom Gingeras described the omnipresence of RNAs ranging from small < 500nt molecules to long transcripts traversing huge regions of the genome. He estimated that on the average 5.2 transcripts covered an annotated gene, but only 1.7 transcripts corresponded to the conventional mRNA. Dmitry Belostotsky revealed novel RNAs expressed from the Arabidopsis genome by using mutant lines defective in the RNA exosome degradation machinery. A similar approach was taken by Alain Jacquier and Torben Heick Jensen, who reported vivid transcription activity around and in particular upstream most active yeast and human promoters. These RNAs are extremely unstable and only reveal themselves when RNA degradation is blocked.

Talks of the second half discussed the transcription termination and RNA degradation mechanisms leading to decay of these unstable transcripts. Steve Buratowski (Harvard

Medical School, Boston, USA) reported that Serine 5 phosphorylation of the C-terminal domain of the large subunit of RNAPII was an important determinant in triggering transcription termination by the so-called Nrd1p-complex. This idea was supported by Domenico Libri (CNRS, Gif sur Yvette, France), who showed that Nrd1p-dependent transcription termination and ensuing degradation by the exosome mainly occurred on short transcription units. Interestingly, Ana Rondon from Nicholas Proudfoot's group (Oxford University, UK) found that in some cases the Nrd1p system could also terminate transcription after longer travels of RNAPII. This supposedly provide a fail-safe mechanism should regular termination not occur. In the final talk by Steve West, also from the Proudfoot lab, it was reported that transcription termination, in addition to its role in disengaging RNAPII from the chromatin template, also serves to generally increase the level of gene expression. It was suggested that termination would release the transcript thereby protecting it from nuclear exosomal decay.

mRNA quality control pathways

The second session was chaired by David Tollervey (first half) and by Torben Heick Jensen (second half). In total, eight speakers focussed on the mechanisms implicated in various mRNA quality control pathways. Allan Jacobson (U. Mass., Worcester, USA) described the selection and analysis of mutants reducing the non-sense suppression activity of a upf1 mutant. Several mutants were identified including ALR1 that encodes a magnesium transporter. This factor modulates the internal magnesium concentration, which in turn affects translation. Other anti-suppressors of upf1 were found to regulate the ALR1 mRNA level. Hervé Le Hir (CNRS, Gif sur Yvette, France) presented a reconstitution analysis of the Exon Junction Complex and its association with Upf factors. This complex forms the heart of a structure involved in NMD in mammalian cells. Andreas Kulozik (U. Heidelberg, Germany) presented a functional analysis of NMD in mammalian cells. The results supported the existence of alternative pathways and a competition of Pab1 and Upf factors for binding eRF3 in the recognition of stop codons triggering NMD. Andrzej Dziembowski (U. Warsaw, Poland) presented a collaborative work demonstrating a new endonucleolytic activity of the yeast exosome. Analysis of mutants implicated this activity in various pathways including the quality control of nuclear transcripts. Then, Allan Jacobson presented on behalf of the absent Stu Petz, results obtained by PTC Therapeutics, a private company developing drugs that would selectively enhance read-through of stop codons recognized as premature and thus increase the production of active proteins. This may be beneficial for patients suffering from numerous genetic diseases, even if only a modest increase in protein production were achieved. Nick Proudfoot then discussed the "punctuation" of eukaryotic genes involving gene looping and the coordination between elements involved in processing and mRNA decay. The next was Oliver Mühlemann (U Bern, Switzerland) who described collaborative results obtained with the group of Torben Heick Jensen demonstrating that Smg6 is endowed with endonucleolytic activity. This activity appears to play an important role in the degradation of NMD substrate in mammalian cells. Finally, Mariangela Morlando reported the coordination between the processing of microRNA and transcription observed in collaboration between the Proudfoot and Bozzoni labs while H  l  ne Gaillard from the Aguilera lab presented an analysis of the formation of cytoplasmic RNA granules observed in yeast following UV treatment.

Structural organization of quality control players

The third session was chaired by Nick Proudfoot. In total, four speakers focussed on the structure of factors involved in mRNA Quality control. Ailong Ke (Lawrence Berkeley National Laboratory, USA) presented an analysis of the structure of the yeast exosome using electron microscopy. Combined with the results of other structural analyses, the results allowed the building of models of interaction of this nuclease with its substrate. Marc Graille (U. Paris Sud, Orsay, France) presented the results of X-ray analyses of various proteins implicated in the recognition of stop codons or related analogues. These factors play key roles in various quality control pathways including NMD and No-Go decay. Esben Lorentzen (MPI, Germany and Birbeck College, London) presented similarly a crystallographic study of a fragment of the Dis3/Rrp44 subunit of the yeast exosome that harbours the exonucleolytic catalytic site of this nuclease. A comparison of substrate-bound Dis3/Rrp44 with bacterial RNase II demonstrated differences in RNA binding. Finally, Laura Milligan from the Tollervey lab (U. of Edinburgh, UK) presented the analysis of a newly identified subunit of the yeast exosome Mpp6, and its role in RNA processing and degradation.

RNA localization and translation

The fourth session was chaired by Michael Kiebler (first half) and by Bertrand Séraphin (second half). In total, eight speakers focussed on another interesting aspect of RNA quality control in polarized cells: RNA localization and its various functions. Research over the last decade or so as well as the talks during the conference clearly showed that mRNA localization is a highly interconnected multistep process, linking RNA biogenesis, nuclear export, cytoplasmic RNA transport, translation and decay (St Johnston, 2005, Nature Rev.). Ilan Davis (Oxford U, UK) reported the investigation of the fate of RNA-containing particles termed ribonucleoprotein particles (RNPs) in *Drosophila* oocytes and embryos. Using time-lapse imaging of mRNA localization, they analyzed the way in which *gurken* mRNA – amongst others – localizes. A particular focus was the role of the microtubule motor protein dynein that seems to have a function in anchoring that is independent of its transport function. Dynein can apparently undergo a switch from a dynamic motor state to a static anchor state. Stefan Hüttelmaier (Halle, Germany) presented interesting evidence on the RNA-binding protein zipcode binding protein 1 (ZBP1) in mammalian cells. In a previous study, while being in the lab from Rob Singer, they have shown that the non-receptor tyrosine kinase Src phosphorylated ZBP1 and thereby modulated its RNA-binding activity, which causes dissociation of the ZBP1 containing RNP. This, in turn, allowed the translation of β -actin at the plasma membrane to occur. In their recent study, they report on the involvement of a MAP kinase as well as a protein phosphatase, PP2B, in this process and present an exciting new model how both processes are interconnected.

A highlight of the meeting was the presentation by Rob Singer (Bronx, New York, USA). By taking advantage of his MS2 imaging system, they studied transcription in living cells. This allowed them to gain unprecedented insight into how transcription occurs from a kinetic standpoint. Joel Richter (U Massachusetts, USA) focused on a recently chosen subject in his lab, translational control of cellular senescence. A marker served the cytoplasmic polyadenylation element binding protein (CPEB), which has important roles in translational regulation in both *Xenopus* oocytes and mouse neurons. He described the characterization

of three additional CPEB-like proteins and provided interesting evidence supporting a role of one of those proteins, CPEB4 in stroke. Finally, Michael Kiebler (Vienna, Austria) focussed on RNA localization into dendrites of polarized rat neurons. Using time-lapse imaging of both GFP-tagged RNA-binding proteins, e.g. Staufen, and Alexa-labelled CaMKII α mRNA, the intracellular fate of RNPs was analyzed. The presented evidence that not only translation is tightly controlled at the synapse, but possibly also RNA stability.

Summary and outlook:

One important feature of this conference was its (limited) size: the total of 52 people attending allowed everybody to talk and exchange ideas not only during the coffee and lunch breaks but also during the nightly dinner. Young students and postdocs got to know each other, explored common territories and had a chance to meet PIs, which are otherwise not accessible to them. PIs gave an overview of the field and discussed open scientific issues as well as possibilities for mobility between laboratories. We are therefore confident that the excellent atmosphere in which this conference took place, was important to further tighten scientific interactions and collaborations. The very good organisation provided by the ESF through Astrid Lunkes and Jackie McLelland, had an essential role in reaching this goal. Overall, this conference – organized and funded by the ESF – allowed us to promote scientific research programmes in Europe in general and to raise general awareness of RNA quality control in particular.