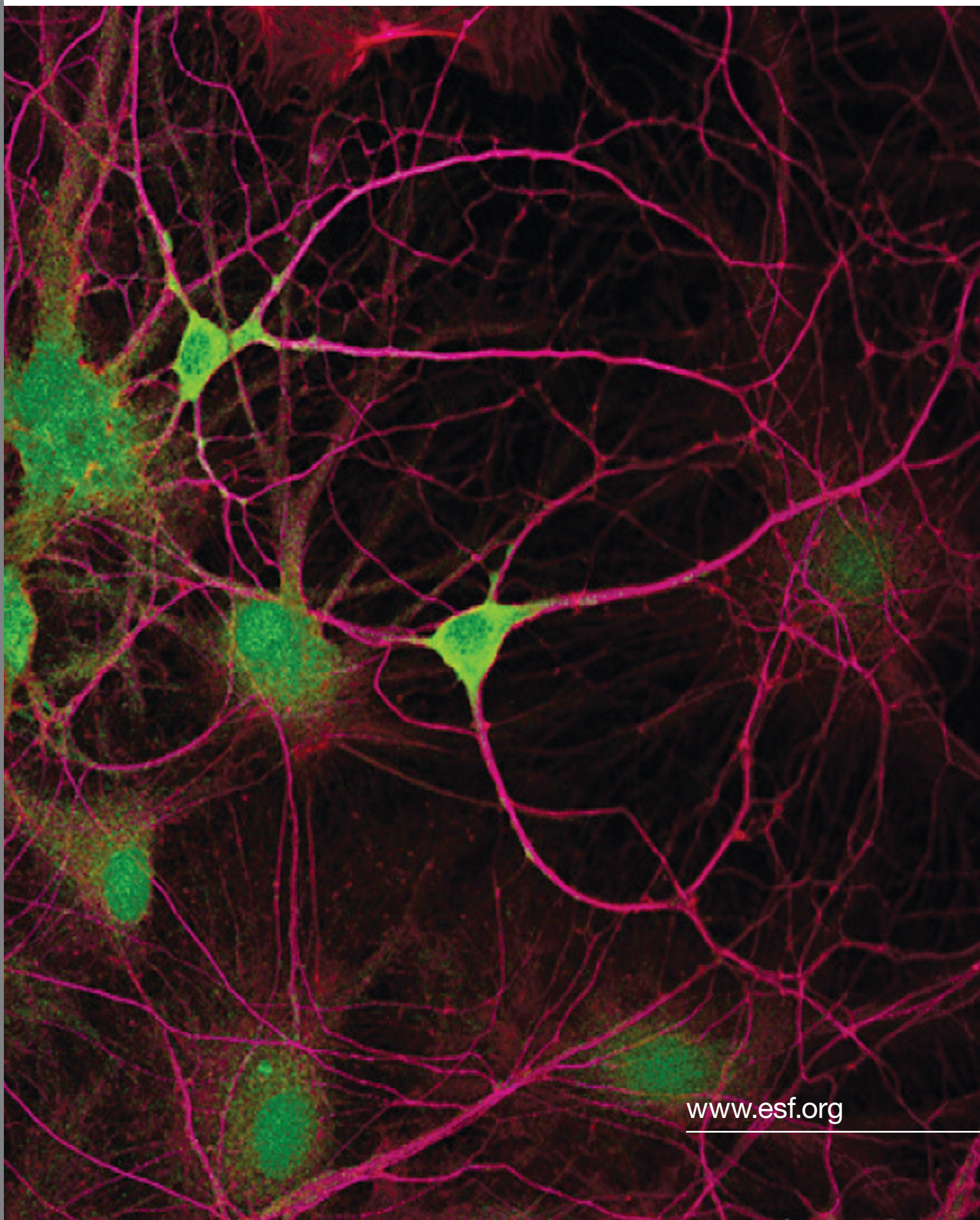
EUROPEAN SCIENCE FOUNDATION

SETTING SCIENCE AGENDAS FOR EUROPE

EUROCORES Programme

RNAQuality

(Quality of Gene Expression – RNA Surveillance)



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Cover Image:
Cover image: Rat hippocampal neurons were cultured until 14 days in vitro and then fixed in PFA. Stau2 (green) and Tubulin (blue) detected by immuno-staining. Phalloidin-staining was used to detect F-actin (red). Lucia Schoderböck, Medical University of Vienna, Austria

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- Biological Chemistry
- Agriculture
- Plant and Animal Biology
- Ecology
- Climate Research
- Earth Sciences
- Glaciology
- Oceanography
- Meteorology

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www.esf.org/rnaquality

Contents

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Austria: Austrian Science Fund (FWF)

Denmark: Danish National Research Council

France: National Centre for Scientific Research (CNRS)

Germany: Max-Planck-Gesellschaft (MPG)

Italy: National Research Council (CNR)

Poland: Ministry of Science and Higher Education

Spain: Ministry of Education and Science (MEC)

Sweden: Swedish Research Council (VR)

United Kingdom: Biotechnology and Biological Sciences Council (BBSRC)

1.5 Support Team at the ESF

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- **Dr Paul Egerton**
  Head of the LESC Unit (2010)
- **Dr Astrid Lunkes**
  EUROCORES Coordinator (2006-2009)
- **Dr Lars V. Kristiansen**
  EUROCORES Coordinator (2009-2010)
- **Ms Jackie McLelland**
  EUROCORES Administrator (2006-2009)
- **Ms Anne-Sophie Gablin**
  EUROCORES Administrator (2009-2010)

2. Description of the RNAQuality Programme

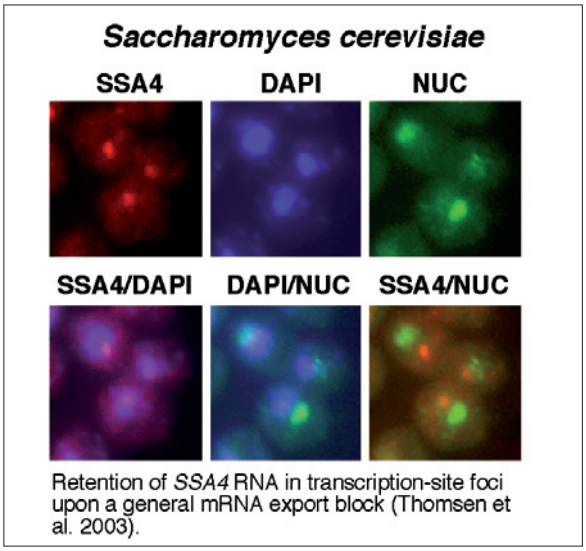
2.1 Background and Scientific Objectives

Following the successful 2006 theme proposal Quality Control of Gene Expression – RNA Surveillance, the RNAQuality EUROCORES programme was launched by ESF in May 2007. This theme was highly rated by both the ESF Science Advisory Board and the Governing Council in that it proposed a top level multidisciplinary research programme consisting of leading European experts investigating key mechanisms in nuclear RNA surveillance linked to transcription – an area that had only recently been fully recognised as integral to normal cell biology.

Cells have developed multiple systems of quality control to ensure that they operate accurately. This also applies to the biogenesis and metabolism of various classes of RNAs, which only recently have been shown to be subjected to stringent surveillance mechanisms. Gene expression was long thought to be regulated almost exclusively at the transcriptional level. However, the growing realisation that post-transcriptional control provides conserved mechanisms by which cells can rapidly change gene expression patterns, together with the discovery of RNA silencing pathways have greatly stimulated interest in the mechanisms of post-transcriptional gene regulation.

Accumulation of abnormally matured mRNA molecules is detrimental to cells given their interference with normal protein synthesis. Surveillance systems that target erroneous RNA molecules for degradation before irreversible cellular damage can occur have therefore evolved. However, surveillance mechanisms not only monitor RNA biogenesis in order to safeguard cells but are now known to also play a role in the post-transcriptional regulation of wild-type transcripts and the elimination of accidentally damaged molecules.

Post-transcriptional processes (e.g. mRNA processing, nuclear export, localisation, surveillance, silencing and turnover) are interlinked by the use of common factors and constitute a complex regulatory network that contributes to cell-type and organism-specific gene expression programmes. Besides providing the means to regulate gene expression at different levels, these interconnected processes also provide opportunities for quality control checkpoints, so that only fully processed and error-free mRNAs are translated into proteins. In addition to monitoring mRNA biogenesis, surveillance mechanisms are probably central to eliminating other classes of defective RNAs, including ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear/nucleolar RNAs (sn/snoRNAs) and cryptic transcripts expressed from intergenic regions.



Retention of *SSA4* RNA in transcription-site foci upon a general mRNA export block (Thomsen et al. 2003).

Although a number of RNA quality control checkpoints in the RNA surveillance pathway have been identified in recent years, the molecular mechanisms behind these checkpoints are still largely unknown. In particular, it is generally unclear how these quality control systems can distinguish between correct and defective RNAs and RNA-protein complexes. Recent studies also indicate that RNA degradation contributes to the stability of the genome by silencing the expression of viruses, transposons and pseudogenes. Moreover, RNA quality control systems are known to modulate clinical manifestations of many genetic disorders, and hence represent promising targets for future therapeutic intervention.

The overarching scientific objective for the RNAQuality programme has been to advance our understanding of processes that act as quality control checkpoints in gene expression and to understand how these are organised at the molecular level. Transcription, processing and degradation of RNA have traditionally been studied as independent processes. During its running period, the RNAQuality programme has initiated a multidisciplinary approach to the topic of RNA biosynthesis that has allowed linkage between molecular/cellular studies of RNA biosynthesis and structural analyses, high-throughput and computational approaches. Through the targeted use of the networking possibilities that is associated with the EUROCORES scheme, the RNAQuality programme has furthermore initiated the establishment of a solid

platform for European researchers from all disciplines of modern life sciences to collaborate in the field of RNA quality control.

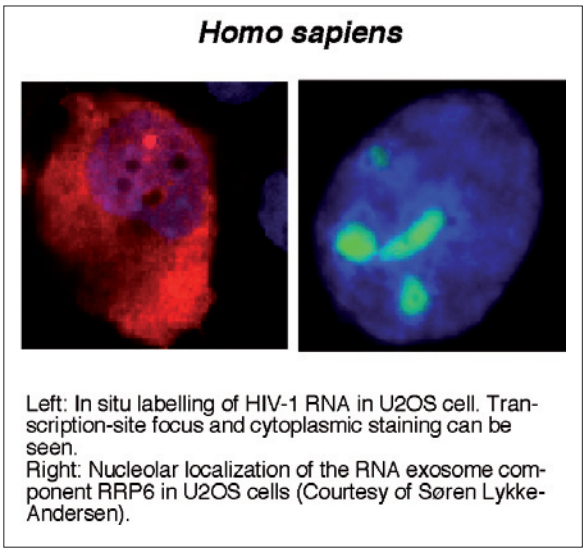
2.2 Detailed Description of the Collaborating Research Areas

2.2.1. Mechanisms of nuclear RNA surveillance

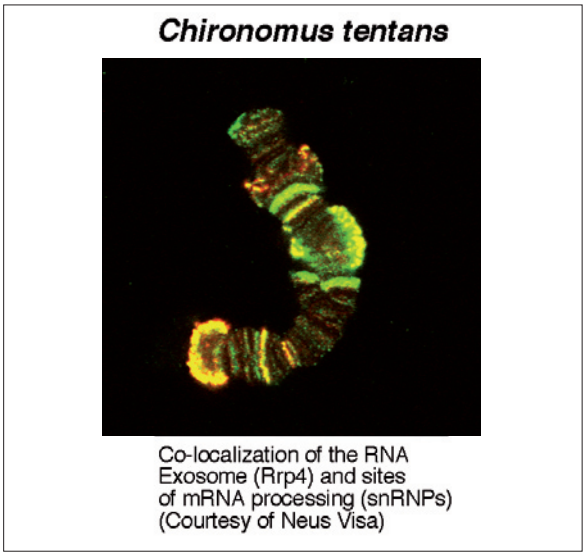
Recent studies have shown that post-transcriptional control and surveillance of gene expression is based on a unified strategy. Despite the notion that the individual components of RNA processing involve only moderate precision, the high quality of the final output of gene expression (proteins) is probably ensured by the overall elaborate and coordinated RNA surveillance mechanisms.

Eukaryotic RNA polymerase II (RNAPII) transcripts are produced and matured in the nucleus. Major events in the biogenesis of most RNAPII-derived RNAs, including the various steps in 3'end processing and RNP formation, occurs simultaneously during transcription. As these processes occur co-transcriptionally, they are tightly linked to the actual process of transcription. Coupling of the processing-packaging systems with transcription in this way therefore sets up an assembly line with a 'built in' quality control (QC) due to the directly linked availability of enzymes and complexes harbouring the required RNA degradative processes.

RNA surveillance mechanisms are manifested through the physical interaction between processing factors and the carboxyterminal domain (CTD) of Rpb1p, the largest subunit of RNAPII, and via interactions between other parts of the transcription complex and RNP maturation factors. Moreover, enzymes and complexes endowed with RNA degradative activity are active players in RNP maturation processes. These complexes actively discriminate between which parts of the transcriptome (or which parts of a transcript) will constitute final mRNA product(s), and which parts will be further be processed and used in gene regulation or be demolished. The tight coupling of RNP formation with transcription increases the efficiency and specificity of this processing-packaging system.



Left: In situ labelling of HIV-1 RNA in U2OS cell. Transcription-site focus and cytoplasmic staining can be seen.
Right: Nucleolar localization of the RNA exosome component RRP6 in U2OS cells (Courtesy of Søren Lykke-Andersen).



Co-localization of the RNA Exosome (Rrp4) and sites of mRNA processing (snRNPs) (Courtesy of Neus Visa)

RNA surveillance pathways operate in both the cytoplasm and the nucleus although cytoplasmic RNA surveillance is frequently dependent on prior nuclear events.

Although the principal focus of the present CRP has been on studies of RNAPII-derived transcripts and consequently surveillance of 'conventional' mRNPs, several projects have been focused on studying RNP surveillance aspects of the synthesis of other RNAs; e.g. miRNAs/siRNAs, CUTs (Cryptic Unstable Transcripts) and IGS (intergenic spacer) transcripts. Further, RNAPII-derived transcripts (e.g. miRNAs/siRNAs, IGS transcripts and perhaps CUTs), in addition to being subject to surveillance themselves, also participate actively as genome surveillance agents by altering chromatin structure and modulating gene expression from specific parts of the genome. Several groups from the present CRP have explored the mechanisms underlying these phenomena, including the overlapping usage of factors involved in multiple RNP surveillance pathways, e.g. the TRAMP and linkage to the exosome complex.

The overall objective for the present CRP was to further our understanding of the mechanisms and rules that

govern RNA surveillance in the nucleus. The individual processes of mRNA synthesis, processing, packaging and degradation are relatively well studied but have traditionally been regarded as independent processes. There is now an increasing realisation that these processes are highly integrated. However, the complexity and speed of these reactions in vivo, together with the analytical challenges posed by the multiple alternative fates of many RNAs, requires the development of many approaches. For this to be achieved, the pooling of expertise, ideas, tools and methodologies from different fields is needed.

2.2.2. Mechanisms of RNA translational control

Translational control and silencing of localised mRNAs is a common mechanism for regulating protein expression in specific cellular subdomains. These events have been shown to play an important role in axes formation, asymmetric cell division, cell motility, and neuronal synaptic plasticity.

Localised mRNAs are usually transported in ribonucleoprotein particles (RNPs) and must be translationally repressed until the RNA reaches its final destination. Interestingly, emerging evidence suggests that translational silencing is directly coupled to RNA transport in mammals. At the molecular level, several translation repressor molecules such as Bruno, CPEB, eIF4AIII, FMRP, and ZBP1 have been described as being involved in RNP-associated silencing. In addition, the double-stranded RNA-binding Staufen 1 is a component of dendritic mRNA transport complexes. Moreover, the DEAD-box protein DDX-6, the mammalian homolog of Me31b, is also present in kinesin-associated RNA granules isolated from rat brain.

This CRP started with a very exploratory and provocative hypothesis suggesting that RNA localisation, mRNA degradation and miRNA-mediated translational silencing might be intimately linked. This was partly based on recent high profile work indicating that not only RNA degradation but also translational silencing is coupled to RNA transport in mammals, since non-coding RNAs, such as microRNAs (miRNAs) and longer regulatory RNAs, e.g. BC1, can repress translation of mRNAs during transport. It has been postulated that this miRNA-guided silencing occurs in another class of RNPs, called processing bodies (P-bodies), which are the major sites of mRNA degradation in both invertebrate and vertebrate cells, and that repressed mRNAs can even be released from P-bodies upon specific signals into the cytoplasm for further translation. Furthermore, compelling evidence suggested that small RNAs together with the bound Ago proteins are responsible for substantial mRNA decay in *Drosophila* S2 cells. This raised the question of whether P-bodies provide a platform for the transport of translationally repressed RNAs, and function as centres that coordinate degradation, translation and localisation.

2.2.3. Disposal of cryptic and aberrant RNAs

In eukaryotic cells, the large multisubunit complex termed the exosome is the main enzyme responsible for 3'-5' RNA degradation. This enzyme is responsible for the degradation of numerous RNA species including aberrant nuclear RNA species such as CUTs and improperly processed tRNAs, ribosomal RNA and snRNA precursors and unspliced pre-mRNAs in the nucleus. In addition, the exosome is involved in the cytoplasm in degradation of defective mRNA that lack a stop codon, those containing a premature stop codon and those provoking ribosome stalling as well as a fraction of non-defective mRNAs. This rather long list of target molecules emphasises not only the central importance the exosome activity plays for eukaryotic cells but also underlines its major contribution to RNA surveillance. Thus, the exosome is involved in the decay of all major types of RNAs in the nucleus (premRNAs, tRNAs, rRNA, snRNAs) once these have been recognised as defective.

The phylogenetically conserved core of the eukaryotic exosome is assembled from 10 polypeptides, nine of which show similarities to the bacterial PNPase and archaeal exosome while the last subunit, Dis3 (also known as Rrp44) shows a strong similarity to bacterial RNase II. Based on sequence similarities and on enzymatic assays performed with isolated recombinant subunits, all subunits of the core eukaryotic exosome are likely endowed with exonucleolytic activity. Furthermore, more recent studies have demonstrated that the exosome acts exclusively as a hydrolytic enzyme with Dis3 carrying the only catalytic site important for in vivo and in vitro exonuclease activity.

Through its diverse activities the exosome plays a central role in the control of RNA quality. The exosome has to target and degrade aberrant RNAs without attacking functional molecules. This discrimination results in part from the action of factors that will bind and/or modify biologically relevant substrates and target them to degradation. Such factors include the Trf/Air poly(A) polymerase and Mtr4 DEAD box helicase in the nucleus and the Ski7 GTpase and Ski2/3/8 complex in the cytoplasm. Although the enzymes and factors involved in these pathways are now relatively well known, the mechanisms leading them to selectively degrade aberrant RNA molecules remain unclear.

Thus far, the exosome is the only exonuclease known to contribute to the degradation of all types of aberrant or damaged RNA that eukaryotic cells need to eliminate even if, in some cases, redundant pathways involving other exonucleases have been described. However,

despite its ubiquitous involvement in RNA quality control, the structural and mechanistic context allowing the recruitment of exosomes for the selective degradation of defective RNA molecules remains poorly understood. In particular, the function of regulatory factors in targeting and/or activating the exosome to degrade aberrant RNAs remains unclear.

In the present CRP a multidisciplinary study of the role of the exosome in RNA quality control pathways was undertaken. This analysis combined molecular biology and biochemical approaches, native mass spectrometry, structural analyses by electron microscopy and small angle X-ray scattering to provide a structural and functional framework allowing the understanding of the role of the exosome in RNA surveillance.

Facts and Figures

Deadline for Applications: 29 May 2006
Funded Collaborative Research Projects (CRPs): Three consisting of 16 Individual Research Projects in 10 different countries.
Duration of Programme: 2007-2010
Budget for research: 3.4 million Euro

2.3 List of Projects

• Nuclear RNA surveillance of genome expression: From yeast to mammals

Torben Heick Jensen (CRP Leader),
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Domenico Libri,
CNRS, Centre de Génétique Moléculaire, Department of Gene Expression, Gif-Sur-Yvette, France.

Nicholas Proudfoot,
Sir William Dunn School of Pathology, Oxford University, Oxford, UK

David Tollervey,
Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, UK

• The role of translational silencing complexes and mRNA degradation factors in RNA localisation in flies and mammals

Michael A. Kiebler (CRP Leader),
Neuronal Cell Biology, Centre for Brain Research, Medical University of Vienna, Vienna, Austria

Gunter Meister,
Max-Planck-Society, Department of RNA Biology, MPI for Biochemistry, Martinsried Germany.

Daniel St Johnston,
Department of Genetics, The Gurdon Institute, University of Cambridge, Cambridge, UK

• Functional and structural dissection of mechanisms targeting the exosome to cryptic and aberrant RNAs

Bertrand Séraphin (CRP Leader),
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Carol Robinson,
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Helen Saibil,
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Andrzej Dziembowski,
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Patrice Vachette,
CNRS, Institut de Biochimie et Biophysique Moléculaire et Cellulaire (IBBMC), UMR 8619, Université Paris-sud, Orsay, France.

2.4 EUROCORES Quality Assurance

2.3.1 Theme selection

New and challenging ideas for EUROCORES Programmes are invited from the scientific community through an annual Call for Theme Proposals. In addition to criteria including scientific quality, novelty and feasibility, the proposals are evaluated on the basis of the requirement for European collaboration: why it is necessary to conduct the programme at a European level and how the programme will strengthen and advance Europe's scientific position in a global context. Each proposal is sent for written external assessment to at least three referees. Based on these reviews, the Science Advisory Board (SAB) recommends which themes are to be further developed, a decision which is then ratified by the Governing Council.

2.3.2 Project selection

The peer review of the Collaborative Research Project (CRP) proposals in a EUROCORES programme such as RNAQuality is a multistage process, including the establishment of an international and independent Review Panel (RP). In response to an open Call for Proposals, outline proposals of about three pages are submitted by a team of applicants (minimum three from three different countries). At that stage, the RP is responsible for the sifting of outline proposals prior to the invitation for submission of full proposals. At the full-proposal stage, each proposal is sent for written external assessments to at least three referees, including referees from outside Europe. Applicants are given an opportunity to reply to the anonymous referee reports. Written referees' assessments and replies by applicants are then considered by the RP with scientific quality being the main selection criterion. The RP makes recommendations for funding of Collaborative Research Projects (CRPs), with prioritisation, which ESF communicates to the EUROCORES Funding Organisations (EFOs).

Following this stringent selection process, three collaborative research programmes (CRPs) were selected for RNAQuality and launched in 2007.

2.3.3 Management Committee

At the time that the Call for Proposals is published, a Management Committee (MC) is established (see page 2 for the RNAQuality MC).

The MC has overall responsibility for the EUROCORES Programme within the guidelines of the EUROCORES Scheme.

The MC can request expert advice from the EUROCORES Scientific Committee, Review Panel or any other ad hoc advisory group.

Members support the EUROCORES review process by nominating a potential Review Panel and external expert referees on behalf of their funding organisation.

Each MC member is responsible for liaising with their funding organisation, including supervision of the funding process for EUROCORES projects within their organisation.

Members may attend all meetings of the EUROCORES programme as observers.

2.3.4 Mid-term and Final Reviews

Each EUROCORES programme undergoes two reviews to evaluate its progress at the mid- and final stages. The aim is to assess scientific cooperation and interactions among the investigators and in the case of a mid-term review to:

- examine the merits of the EUROCORES programme and its potential
- provide recommendations for the second part of the programme

and in a final review to:

- examine the merits of the EUROCORES programme and the lessons there are to be learned for potential follow-up initiatives.

The programme is assessed using the following criteria:

- Novelty/Originality: The most innovative/original scientific contribution of each CRP to the programme and to the relevant field of research.
- Multidisciplinary research: How each CRP is working

towards (or achieving) multidisciplinary research.
- Collaborative research: Results obtained within the CRP during this reporting period that would not have been achieved (or would have taken longer to achieve) in an individual project.
- European-added value: The European dimension given to national funding (e.g. building up the ERA; developing a critical mass of expertise; addressing issues of scale and scope). For CRPs involving partners outside Europe: a clear example illustrating their added value to the programme and their contribution to the relevant field of research in Europe.
- Relevance to the Call: Achievement most relevant to the Call.

The RNAQuality programme received overall very positive mid-term and final evaluations. The consensus mid-term report applauded the programme for bringing together a 'particularly innovative and active group of scientists'. The achievements that were particularly highlighted in the mid-term and final reports included the impressive high level of inter- and intra-CRP collaboration as demonstrated in several joint CRP publications and the overall impressive list of papers in highly ranked journals.

The programme was particularly lauded for its excellent use of research training opportunities for the associated young trainees, which involved exchange visits, schools and workshops dedicated to graduate student interaction. Further, the general focus on collaboration with open exchange of information was mentioned in these reports.

2.3.5 EUROCORES acknowledgements

To promote the EUROCORES programme and the national funding organisations who support it (and prior to 2008, the European Commission), all publications, posters, websites and other dissemination outputs are required to be clearly identified as being programme-funded or co-funded. This is an important indicator for monitoring the outputs of the programmes, particularly peer-reviewed publications.

3. Highlights of the RNAQuality Collaborative Research Projects (CRPs)

3.1 Nuclear RNA surveillance of genome expression: From yeast to mammals (CRP I)

Principal Investigators

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University of Aarhus, Denmark
Andrès Aguilera
University of Seville, Spain
Irene Bozzoni
CNR-IBPM, University of Rome 'La Sapienza', Italy
Alain Jacquier (Co-PI)
Institut Pasteur, Paris, France
Domenico Libri
GM – CNRS, Gif-sur-Yvette, France
Nicholas Proudfoot
Oxford University, United Kingdom
David Tollervey
University of Edinburgh, United Kingdom
Neus Visa
Stockholm University, Sweden

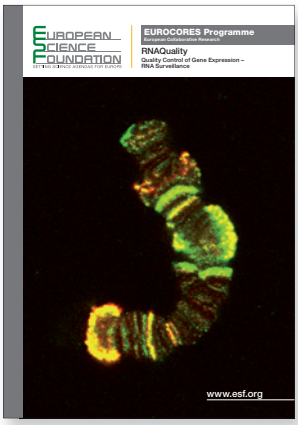
Funding Organisations

Denmark: Forskningsrådet for Natur og Univers (FNU)
France: Centre National de la Recherche Scientifique (CNRS)
Italy: Consiglio Nazionale delle Ricerche (CNR)
United Kingdom: Biotechnology and Biological Sciences Research Council (BBSRC)
Spain: Ministerio de Educación y Ciencia (MEC)
Sweden: Vetenskapsrådet – medicine (VR)

The objective of the collaborative work within this CRP was to study the mechanisms and rules governing RNA surveillance in the nucleus, about which relatively little is known. The individual processes of mRNA synthesis, processing, packaging and degradation are relatively well studied, but have traditionally been regarded as independent processes. There is now an increasing realisation that these processes are highly integrated. However, the complexity and speed of these reactions in vivo, together with the analytical challenges posed by the multiple alternative fates of many RNAs, represent new frontiers that require multidisciplinary collaboration and development of new scientific tools to be investigated.

The eight participating labs have collectively addressed six related research areas that overall corresponded to the objective of the CRP outlined initially. These involved:

1. **Characterisation of factors involved in nuclear surveillance**
   The Tollervey and Jacquier labs used genetic approaches to identify the in vivo substrates for TRAMP and Mpp6p, two recently identified exosome co-factors, using a genomic scale approach in yeast. Furthermore, the Libri team studied the mechanistic aspects of TRAMP function and comparative functional studies on the mammalian TRAMP were carried out by the Bozzoni lab. The Visa lab carried out localisation studies in the Balbiani ring (BR) system of Chironomus tentans and the Visa and Bozzoni teams used biochemical approaches in *Drosophila* and human cells, to identify nuclear exosome subpopulations with specific composition and/or substrate specificity.



2. **Surveillance of aberrant RNP formation**
   The THO/Sub2p complex is a good model system in which to study quality control. The Jensen lab has used this system to focus on the links between 3'end processing and QC and its sensitivity to mutations in specific areas of the THO/Sub2p system. The initial pre-mRNA processing assays were followed by biochemical characterisation of the composition of 3'end processing complexes and in vivo analysis in collaboration with the Libri lab (see below). In parallel, the Aguilera lab in collaboration with the Jensen and Proudfoot labs carried out a search for synthetic lethal mutations for rrp6 and rna14, thereby characterising the transcription properties of the mutants both in vivo and in vitro. Further, the Aguilera lab randomly mutagenised selected components of the THO and found mutations that separate the putative functions of THO in transcription and surveillance. Complementing these assays, the Libri lab carried out chromatin immunoprecipitation (ChIP) and microarray experiments to characterise chromatin defects related to THO function.

   The Jacquier lab analysed how different mutations in the mRNP processing/assembly machineries affect nuclear retention and degradation on a genomic scale. Using studies in yeast and insects systems, disturbances in the recruitment of THO components

to nascent transcripts in different mutant backgrounds was analysed using immunofluorescence and FISH by the Aguilera, Jensen and Libri labs (each lab using different reporters and endogenous genes). Further, the effects of THO depletion and overexpression in human cells was analysed in collaboration between the Aguilera and the Bozzoni labs.

The Bozzoni group additionally undertook studies aimed at understanding the loading of processing factors onto the miRNAs and the packaging of miRNAs into functional miRNP complexes. This project aims at identifying surveillance checkpoints able to sense aberrant miRNP assembly.

3. Surveillance of transcription termination

To further understand the termination process, the Proudfoot lab investigated whether recently identified co-transcriptional cleavage (CoTC) sequences in RNAPII termination regions of protein-coding genes are potential entry sites for exonucleases such as the exosome and Xrn2.

In parallel studies, the Bozzoni group analysed the involvement of hXrn2 in transcriptional termination of miRNA genes and worked on characterisation of the factors involved in the processing of primiRNAs and in surveillance of miRNA biogenesis, with particular focus on the study of 3'end cleavage and on the importance of the cleavage site for correct miRNP assembly.

4. Surveillance of cryptic transcription

Cryptic Unstable Transcripts (CUTs) display half-lives of less than two minutes, whereas other types of non-coding transcripts derived from RNAPII are stable. The Libri lab has identified a CUT sequence element that targets RNA to degradation through a mechanism that requires the Nrd1 and Nab3 proteins. With a combination of genome-wide in silico analyses and experimental validation, the Libri and Jacquier labs have studied the coupling between Nrd1p/Nab3p termination and the exosome by generating a 'pre-terminated' RNA and assessing whether it is degraded in a Nrd1p/Nab3-sensitive manner. Additionally, the recruitment of surveillance factors to non-coding RNAs was studied in the Libri and Tollervey labs using ChIP to study the co-transcriptional recruitment of Nrd1p/Nab3, TRAMP and exosome to different types of cryptic transcripts.

Factors involved in CUT surveillance and how mutations in these affect the nuclear retention and/or degradation of CUTs were studied in the Jacquier lab. This work helped identify functional interactions between the different steps of CUT biogenesis and surveillance.

5. Identification of new surveillance pathways

The Jacquier lab analysed functional interactions between different steps of RNA maturation and surveillance pathways by applying a new genetic method for quantitative analysis on a genomic scale. Specific pathways components were identified and further analysed by the Jacquier lab in collaboration with the Libri, Tollervey, and Jensen teams.

In a search for surveillance pathways linked to RNAPII, the Jensen lab identified rpb1p mutations associated with mRNA nuclear retention phenotypes. Following these discoveries genetic methods were applied to characterise the defects of the rpb1p mutants and in collaboration with the Jacquier lab, these were tested for genetic interactions on a genomic scale.

6. Functional relationships between surveillance pathways and chromatin status

Based on the observation that the levels of non-coding RNAs associated with silenced chromatin are elevated in cells defective in components of the TRAMP/exosome system, the Proudfoot lab studied the synthesis of intergenic transcripts which may act as substrates for siRNA production. Supporting this work, the Tollervey lab used ChIP to study the recruitment of TRAMP and exosome to IGS transcripts, and tested candidate RNA-binding factors. To assess the requirement for the TRAMP/exosome system in repressive chromatin formation, de-repression of reporter constructs integrated in either the rDNA ISG or sub-telomeric region was furthermore evaluated. In parallel experiments, the Tollervey lab also studied interactions between Trf4p and condensin in collaboration with the Jacquier lab, and investigated how TRAMP might be involved in the release and degradation of nascent pre-rRNAs at stalled replication forks.

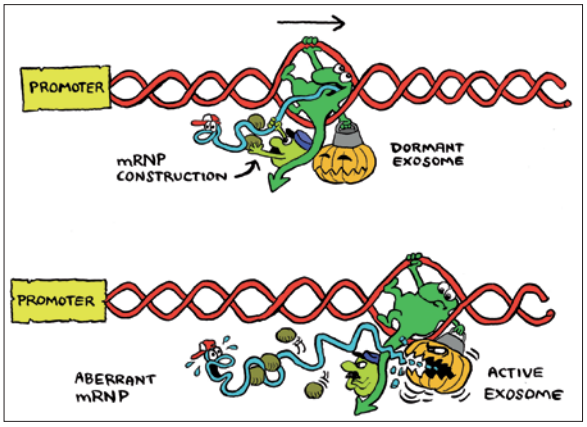


Figure 2. Nuclear RNA surveillance processes are often coupled to the act of gene transcription. A schematic view of this concept is presented above. Layout courtesy of Ebbe Sloth Andersen.

3. Highlights of the RNAQuality Collaborative Research Projects (CRPs)

Expressed opinion by the scientists in CRP I

The international linkage of the CRP has been of tremendous value in that it has brought together a critical mass of researchers with related but distinct research foci. Thus, CRP cooperation has greatly increased the quality of research within the group.

Networking within the programme provided strong synergistic effects and strongly helped to further establish the European teams as world leaders in the field. The RNA Quality programme has played an important role in stimulating and coordinating Europe-wide research into an important and rapidly evolving field of research. These networks, in which groups are free to develop the best research and collaborative projects, are highly preferred over the traditional, EU-funded networks in which a rigid, administrative interpretation of the programme can inhibit the development of new ideas and innovations.

Numerous collaborative projects will continue beyond the formal termination the programme.

Selected Publications

- **Submitted/under revision**

Cacchiarelli D, Martone J, Girardi E, Cesana M, Incitti T, Nicoletti C, Santini T, Sthandier O, Auricchio A, Musarò A and **Bozzoni I**. Dystrophin-dependent epigenetic reprogramming of muscle miRNA expression, submitted.

Camblong J, S-M Tan Wong, JB Zaugg, Z Xu, NM Luscombe, LM Steinmetz, and **NJ Proudfoot** . Gene loops promote transcription fidelity. *Nature,* under revision

Domínguez-Sánchez M, Sáez C, Japón MA, **Aguilera A** and Luna R. Differential expression of THOC1 and ALY mRNP biogenesis/export factors in human cancers. Under revision.

Eberle AB, Hessle V, Helbig R, Dantoft W, Gimber N, **Visa N**. Splice-site mutations cause Rrp6-mediated nuclear retention of the unspliced RNAs and transcriptional down-regulation of the splicing-defective genes. Under revision.

Gullerova M, Moazed D, **Proudfoot NJ.** Convergent orientation of RNA interference genes autoregulates fission yeast heterochromatin. *Nature,* under review

Mapendano CK, Lykke-Andersen S, Kjems J, Bertrand E and **Jensen TH.** Crosstalk between mRNA 3'end processing and transcription initiation. *Mol. Cell* (2010), revised manuscript in review.

Silverstein RA, González de Valdivia E, **Visa N**. The incorporation of 5-fluorouracil into RNA affects the ribonucleolytic activity of the exosome subunit Rrp6. Under revision.

Wijayatilake H, Tan Wong S-M, **Proudfoot NJ.** Promoter retention and polymerase pausing mediate intra-genic looping during mammalian transcription. *Nature SMB,* under revision.

- **2011**

Mischo HB, Gomez Gonzales, Rondon AG, Wei W, Steinmetz LM, Aguilera A, **Proudfoot NJ.** Yeast Sen1 helicase protects the genome from transcription associated instability. *Mol. Cell*.

- **2010**

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3.2 The role of translational silencing complexes and mRNA degradation factors in RNA localisation in flies and mammals (CRP II)

Principal Investigators

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Funding Organisations

Austria: Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (FWF)
Max-Planck-Gesellschaft: (MPG)
United Kingdom: Biotechnology and Biological Sciences Research Council (BBSRC)

Translational control of localised mRNAs is a common mechanism for regulating protein expression in specific cellular subdomains which plays an important role in axes formation, asymmetric cell division, cell motility, and neuronal synaptic plasticity. Localised mRNAs are usually transported in RNPs and are thought to be translationally repressed until the RNA reaches its final destination.

This CRP started with a very exploratory and provocative hypothesis suggesting that RNA localisation, mRNA degradation and miRNA-mediated translational silencing might be intimately linked. This was partly based on recent high profile work indicating that RNA granules at the structural and functional levels interact with somatic P-bodies and that miRNAs are not required for mRNA localisation in the *Drosophila* oocyte. However, initial work found that neuronal RNA granules are clearly separated from P-bodies and much more dynamic and heterogeneous than previously anticipated. Additionally, the St Johnston lab showed that the assumption that miRNAs do not affect mRNA localisation in *Drosophila* was incorrect. Unexpectedly, these mutants were found to disrupt mRNA localisation through an indirect pathway.

Confronted with this new information, the hypothesis for this CRP was modified to one indicating that RNA localisation involves dynamic and heterogeneous RNA granules in both *Drosophila* embryos and mammalian neurons. The work to investigate this hypothesis involved two strategies. First, using time-lapse videomicroscopy in *Drosophila* embryos, the St Johnston lab succeeded in elucidating the underlying mechanism of *osk* and *bicoid* RNA transport. This work demonstrated that whereas *osk* mRNA particles have a slight but significant bias towards the posterior, the *bicoid* mRNA particles do not show a comparable bias to the anterior. This discovery led the Kiebler lab to evaluate whether transport of dendritically localised mRNAs, e.g. β-*actin*, *CaMKII*α, are more comparable to the *osk* or the *bicoid* mRNA particles in neurons.

Using a modified MS2 system in mammalian neurons, transport of the *CaMKII*α and β-*actin* mRNA we did not find a biased walk, but we identified different transport velocities of anti-retrograde versus retrograde transport along bi-directional microtubules in dendrites. Furthermore, our results strongly suggest that RNA granules are not just mere P-bodies or polar granules, but rather very dynamic and heterogeneous structures that might transiently interact with some selective proteins, previously considered to be robust P-body markers.

A second major finding in this CRP was the discovery that mammalian Ago proteins are subject to phosphorylation and that synaptic activity critically affects phosphorylation of Y529 in Ago2, thereby yielding rapid disassembly (possibly degradation) of Ago2 particles in dendrites at the synapse. This suggests a mechanism for regulation of RNAi function in primary neurons and at the activated synapse. It is therefore currently being investigated whether Ago proteins are degraded upon synaptic activity and whether this may affect miRNA-mediated translational control at synapses thereby contributing to the molecular and morphological reorganisation of synapses during synaptic plasticity. Although the cascade of events downstream of neural depression that lead to modifications of Ago2 are largely unknown, we have identified for the first time a correlation between activity and the function and stability of a major component of the RNAi pathway, thus opening a new and exciting line of research.

In summary, the collaborative research within this CRP has provided detailed insight into how RNA localisation, mRNA degradation and miRNA-mediated translational silencing might possibly be linked. Clearly, RNA granules are not just P-bodies (or polar granules in *Drosophila*), but much more dynamic and heterogeneous than previously anticipated. However, we are very excited about this dynamic and interesting link. We are currently exploring future funding possibilities for our team outside the European framework to continue and foster our collaborative efforts, whether this will be future ESF or HFSP networks.

Selected Publications

- **Submitted**

Mikl M, Vendra G, and **Kiebler MA.** Independent regulation and localisation of *MAP2*, *CaMKII*α *and* β-*actin* RNAs in low copy numbers. Submitted.

Rüdel S, Wang Y, Lenobel R, Körner R, Hsiao HH, Urlaub U, Patel D, **Meister G.** Phosphorylation of human Argonaute proteins affects small RNA binding. Under revision.

- **2010**

Dölken L, Malterer G, Erhard F, Kothe S, Friedel CC, Suffert G, Marcinowski L, Motsch N, Barth S, Beitzinger M, Lieber D, Bailer SM, Hoffmann R, Ruzsics Z, Kremmer E, Pfeffer S, Zimmer R, Koszinowski UH, Grässer F, **Meister G,** Haas J (2010). Systematic analysis of viral and cellular microRNA targets in cells latently infected with human gamma-herpes viruses by RISC immunoprecipitation assay. *Cell Host Microbe,* 7:24-34.

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Mikl M, Vendra G, Doyle M and **Kiebler MA** (2010). RNA localisation in neurite morphogenesis and synaptic regulation – current evidence and novel approaches (Review). *Journal of Comparative Physiology – A (sensory, neural, behavioral physiology),* 196:321-34.

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Konecna A, Heraud JE, Schoderboeck L, Raposo AA, **Kiebler MA** (2009). What are the roles of microRNAs at the mammalian synapse? *Neurosci. Lett*. 4; 466(2):63-8. Epub 2009 Jun 21.

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- **2008**

Ender C, Krek A, Friedländer MR, Beitzinger M, Weinmann L, Chen W, Pfeffer S, Rajewsky N, **Meister G** (2008). A human snoRNA with microRNA-like functions. *Mol. Cell,* 32:519-28.

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3.3 Functional and structural dissection of mechanisms targeting the exosome to cryptic and aberrant RNAs (CRP III)

Principal Investigators

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Helen Saibil
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Funding Organisations

France: Centre national de la Recherche Scientifique (CNRS)
Poland: Ministerstwo Nauki i Szkolnictwa Wyższego
UK: Biotechnology and Biological Sciences Research Council (BBSRC)

The exosome has been implicated in most decay pathways involved in the removal of aberrant and non-functional RNA molecules. Those include elimination of Cryptic Unstable Transcripts (CUTs), aberrant tRNAs, nonspliced pre-mRNA as well as some snRNAs and rRNAs in the nucleus. In the cytoplasm, the exosome has been show to degrade non-functional mRNAs containing a premature stop codon (NMD), those lacking a stop codon (NSD) or those provoking ribosome stalling (no-go decay). The exosome thus plays a central and essential role in the control of RNA quality.

The exosome consists of a core unit assembled from 10 different proteins to which nuclear and cytoplasmic specific subunits are associated. A key requirement for the exosome is its accurate targetting and degradation of aberrant RNAs without attacking functional

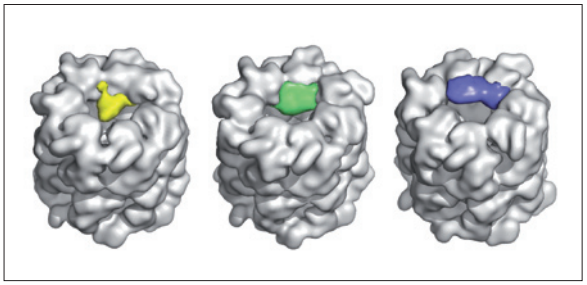

Figure 3. Structural representation of the eukaryotic exosome with colour labelling of different active sites.
Courtesy H. Saibil.

3. Highlights of the RNAQuality Collaborative Research Projects (CRPs)

RNA molecules. Some of this discriminatory capacity results in part from the action of factors that will bind and/or modify biologically relevant substrates and target them to degradation. Such factors include the Trf/Air poly(A) polymerase and Mtr4 DEAD box helicase in the nucleus and the Ski7 GTpase and Ski2/3/8 complex in the cytoplasm.

Despite a good understanding of the main players in exosome degradation, structural and mechanistic data explaining how this machinery operates to selectively degrade aberrant RNA in the nucleus and cytoplasm of eukaryotic cells are still lacking.

The main novel and original scientific outputs from this CRP involved:

- Identification of endonucleolytic activity within the exosome. The eukaryotic exosome was until then thought to be active only as an exonuclease.
- Structure of the yeast apo and RNA-bound exosome complexes by cryo-electron microscopy defining the path of the substrate RNA. In the substrate-bound structure, extra density representing the RNA is visible at the entry site of the exosome channel. At the exit site of the channel, the cryo-EM map suggests a prominent domain movement in Rrp44 that probably serves to position the nucleic acid inside the channel. These data indicate a substrate-threading mechanism via the central channel of the eukaryotic exosome.
- Analysis of the role of Hbs1-Dom34 factors implicated in the NGD and NRD RNA quality control pathways that implicate exosome-dependent RNA degradation. SAXS analyses and functional assays in vivo complemented an X-ray structure obtained by a group outside the CRP.

These results involved for each case, collaboration within and between the participating CRPs.

Selected Publications

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Kafasla P, Morgner, N, **Robinson CV**, Jackson RJ. Polypyrimidine tract binding protein stimulates the poliovirus IRES by modulating eIF4G binding. Submitted**.**

Hélène M, Topf M, Clare DK, Ebert Ju, Bonneau F, Basquin J, Drazkowska K, Tomecki R, **Dziembowski A**, Conti E, **Saibil HR,** Lorentzen E. Cryo-EM structures of the yeast exosome in the unbound and RNA-bound conformations. Submitted**.**

Tomecki R and **Dziembowski** A. Novel endoribonucleases as central players in the general pathways of eukaryotic RNA metabolism. ***RNA***, in press

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Tomecki R, Drazkowska K and **Dziembowski A** (2010). Mechanisms of RNA degradation by the eukaryotic exosome. ***Chembiochem***. 11:938-45.

Gas-Lopez, M. and **Séraphin**, **B**. Twins take the job. ***EMBO J.*** Submitted.

van den Elzen AMG, Henri J, Lazar N, Gas-Lopez M, Durand D, Lacroute F, van Tilbeurgh H, **Séraphin B,** Graille M. Structural and functional dissection of the Dom34-Hbs1 complex reveals independent functions in NGD and NRD. Submitted.

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Lebreton A, Tomecki R, **Dziembowski A**, **Séraphin B** (2008). Endonucleolytic RNA cleavage by a eukaryotic exosome. ***Nature,*** 456:993-6.

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4. Networking and Dissemination Activities

Networking and Dissemination activities are key characteristics of any EUROCORES Programme including RNAQuality. For the EUROCORES scheme, the aim of adding these is to facilitate and encourage scientific collaboration and diffusion of information within and across the participating Collaborative Research Projects (CRPs). Networking activities are therefore very flexible and can be tailored by the collaborating scientists to fit the needs of a particular scientific community. Dissemination activities are likewise also very flexible and are intended to facilitate communication about the programme to non-participating but related scientific communities as well as to society as a whole.

Networking activities

Collaborative networking activities aim at bringing together scientists from all scientific domains of the EUROCORES programme in order to facilitate general interaction, and to discuss, plan and implement future collaboration.

Typical examples of these activities are:

- Working group meetings, seminars, workshops, symposia, conferences
- Summer Schools
- Training programmes and specialised courses (graduate-level and continuing-education)
- Exchange visits.

Dissemination activities

Dissemination within a EUROCORES programme serves the purpose of raising awareness of and diffusing the results of the programme. These activities may include:

- Leaflets, posters, publications, books, exhibition booth at conferences
- Organising programme-relevant scientific sessions at larger conferences (when the EUROCORES programme is not directly involved in the conference as a main or co-organiser of the event)
- Dissemination of travel grants to support an active participation at conferences (organised outside the EUROCORES programme), while promoting the EUROCORES scheme in general and disseminating the achievements of the programme in particular.

This section provides an overview of the main networking and dissemination activities of the RNAQuality programme.

• Mini-Symposium on RNAbiogenesis and Quality Control, 18 September 2007, Aarhus University, Denmark

RNA biogenesis and quality control has recently emerged as an important field of research. Surprisingly, eukaryotic cells degrade the majority of RNA produced, and a considerable portion of discarded species are rejected by quality control machineries. The importance of such control is exemplified by studies reporting clinical manifestations of failure to execute adequate RNA surveillance. At the symposium, both structural and functional aspects of RNA biogenesis and quality control were addressed; as was the issue in both procaryotic and eukaryotic cells.

The aim of this initial symposium was to provide an efficient platform for establishing collaborations across the participating CRPs within the RNAQuality programme, and moreover, to establish links to laboratories within the related EuroDYNA EUROCORES programme. The meeting was attended by approximately 200 people: Students and postdocs from the participating laboratories as well as interested employees from Danish universities. Scientific issues as well as possibilities for mobility between laboratories were discussed.

The symposium brought together participants from two different CRPs within the RNAQuality programme as well as Dr David Shore from the EuroDYNA programme. In addition, four external experts were invited as keynote speakers.

• RNAQuality First Conference, 11-13 June 2008, Granada, Spain

The initial steps to organise this first RNAQuality conference were taken during the programme kick-off meeting in Strasbourg, France on the 11 May 2007. Here, the three CRP Project Coordinators, Bertrand Séraphin, Torben Heick Jensen and Michael Kiebler, met with ESF

Figure 4. The Alhambra, Granada, Spain

programme officers to discuss future networking possibilities. The consensus was that a first conference should help bridge the diverse topics represented within the three collaborating teams: RNA quality control with a special emphasis on the exosome on one hand and RNA localisation and translational control on the other.

The conference brought together participants from all three CRPs of the RNAQuality programme. In addition, 15 external experts representing the different topics of the programme attended as speakers. The symposium was attended by 52 scientists ranging from early career students and postdocs to world-renowned international experts. From an RNAQuality standpoint, the conference achieved several key goals. First, it facilitated new collaborations across individual CRPs within the RNAQuality programme. Second, it helped map the current status of both the RNA quality and RNA localisation fields; 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 organisation of quality control players.
4. RNA localisation and translation.

The small format of the conference (52 scientists) provided ideal conditions for networking and exchange of ideas. Young students and postdocs got acquainted, explored common territories and had a chance to meet more experienced scientists. Plenary lectures from the leading Principal Investigators (PIs) provided general overviews of the collaborating areas, which led to open discussion and exchange. Overall, this conference was the initial success which helped frame the remainder of the programme. Press releases available at http://www.esf.org/rnaquality

• PhD Summer School on Structure and Function of mRNP, 4-8 August 2008, Aarhus University, Denmark

The summer school was attended by approximately 30 PhD students and post docs – all affiliated to the leading RNA/RNP laboratories in Europe, Australia and Russia. The structure of the workshop was based on scientific discussions; i.e. the lecturers of the day would give an introductory presentation of the session topic after which a lively discussion would start. All students had previously received original papers and reviews on the respective topics and, to secure a high academic level in the discussion, students had prepared at least five questions per lecture. This precaution proved absolutely unnecessary and after only few minutes of lecturing all teachers engaged in lively and high quality conversations with the students, and there was a clear impression that all parties enjoyed the straightforward and frank exchange of ideas/information.

Some lectures were methodologically based (Séraphin, Carmo-Fonseca, Kiebler, Andersen, Brodersen) and others rooted in scientific concepts (Moore, Visa, Jensen, Jankowsky). It appeared that both versions were well received by the student participants. To finalise each day, the two lecturers would give an afternoon talk on a timely practical project from their laboratory.

The summer school was generally perceived as a key success of the programme, which helped further strengthen the personal and scientific bonds between scientists of the programme.

• Workshop RNAQuality 2009, 13-15 May 2009, Gif-sur-Yvette, France

Within this three-day workshop every group from the RNAQuality EUROCORES was encouraged to contribute with their hottest stories from the laboratory. To extend this in an inclusive manner to encompass the integrated view of European research on eukaryotic mRNA quality control, four external groups were invited (Edouard Bertrand from Montpellier, Oliver Muhlemann from Bern, Françoise Stutz from Geneve, Saverio Brogna from Birmingham).

A total of 57 people attended this meeting with a perfect mix of approximately 25% of PIs and 75% of PhD students and postdocs. On average, each PI came with two or three students/postdocs.

The workshop included 20 presentations by either a PI or student/postdoc. The talks were grouped into small sessions covering the following topics:

- coupling of pre-mRNA synthesis and processing
- mRNP modelling and transport

- RNA decay including action and targets of the exosome
- nonsense-mediated mRNA decay
- short and long non-coding RNAs.

All discussions were based on the oral presentations. Every lab had a 40-minute slot with 30 minutes of presentation and 10 minutes for discussion. These long talks were appreciated by the participants as they provided enough time for an introduction and experimental approaches as well as for discussion of novel ideas. All sessions were chaired by students from different groups, which helped to introduce them to the community.

• RNAQuality Students' Workshop, 29-30 April 2010, Strasbourg, France

The idea behind this networking activity was to organise a workshop that would give an opportunity to PhD students and postdocs from related fields (RNA quality) to present and discuss their work in progress. This goal was well met: during the day and a half, 21 out of 25 participating students and postdocs presented their work in oral presentations of 20 minutes. Another aim of the meeting was for the participants, in the absence of PIs, to engage in low-threshold 10-minute discussions following each talk.

In summary, the student workshop was very successful, which was also confirmed by the positive feedback from participants. A student workshop is a good way to provide a platform for students and postdocs to present their work, and especially for recently starting students to obtain some experience in doing so. Moreover it is a good way for students and postdocs to learn about what other labs in their field are working on.

Figure 6. Participants of the workshop

Figure 7. View of Vienna, Austria

• RNAQuality Control Final Conference, 10-13 May 2010, Vienna, Austria

It has long been recognised that damaged or improperly folded proteins are quickly targeted for degradation. In contrast, it has only recently become clear that defective mRNAs are also actively directed for rapid degradation. During the running time for the RNAQuality programme, new discoveries within this and related fields have furthered our understanding considerably.

Given the increasing insight and interest from diverse scientific areas of RNA-related research, the coordinators of the RNAQuality programme proposed to organise a final workshop/conference on this topic. In order to broaden the scope and interest, the workshop was established in collaboration between EMBO and ESF. The resulting EMBO-ESF workshop was held in Vienna from 10 to 13 May 2010.

Thirty speakers were invited to present their work at the workshop. Speakers were chosen to represent as much as possible within the dynamic research area. Their distribution by continent of origin and male/female distribution is detailed in Table 1.

Table 1. Speakers by gender and continent

	Female	Male
Europe	8	13
USA/Canada	2	5
Asia	0	1

Due to organisational constraints (housing, meals) and to maintain a meeting size allowing a maximum of contact and discussion between participants, a total of 65 applicants were selected from 110 received applications.

Attendees ranged from PhD students to PIs and included many postdoctoral trainees. Further, three participants were editors of scientific journals (*The EMBO Journal, Nature and Nature Cell Biology review/ Nature Structural and Molecular Biology*) underlining major interest in this area.

It was a considerable strength of the meeting to integrate attendees from different fields that usually do not attend the same events. The limited size of the meeting together with sufficient time apart from the oral presentations gave opportunities for students and postdocs to approach and interact with PIs.

• RNAQuality Control workshop,
1-2 October 2010, Châteaux de la Loire, France

The workshop accommodated a total of 50 students and 10 PIs including invited speakers (Gregers R. Andersen (Aarhus), Sabine Ruedel (Gunter Meister lab, Regensburg) and Maria Gas-Lopez (Bertrand Séraphin lab, Strasbourg). The workshop consisted of plenary talks by the invited keynote speakers as well as 30 short talks by selected students. The topics discussed included the latest developments in the field of RNA quality control. Different biological systems ranging from yeast to mammals were included as well as presentations of cutting edge high throughput approaches applied to the studies of RNA quality.

Meetings programmes and more details are available at www.esf.org/rnaquality

5. Related ESF Activities

During the RNAQuality networking phase, two different ESF science activities have had specific relevance to this EUROCORES programme. These are the Forward Look entitled: 'RNA World: a new frontier in biomedical research' and the research networking programme (RNP) on frontiers of functional genomics (FFG).

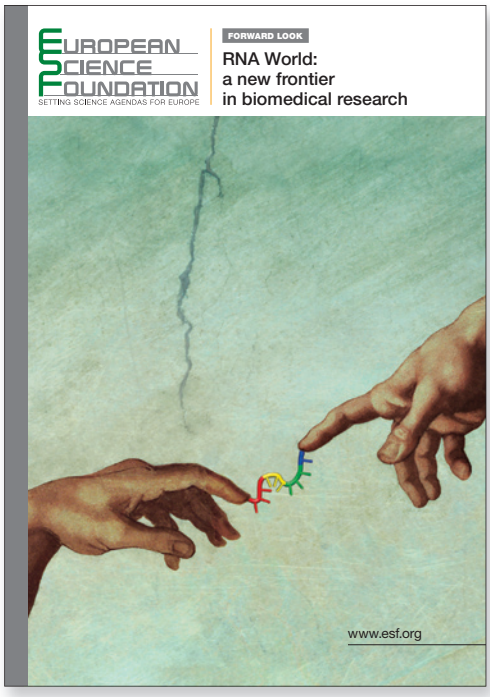
5.1 RNA World: a new frontier in biomedical research

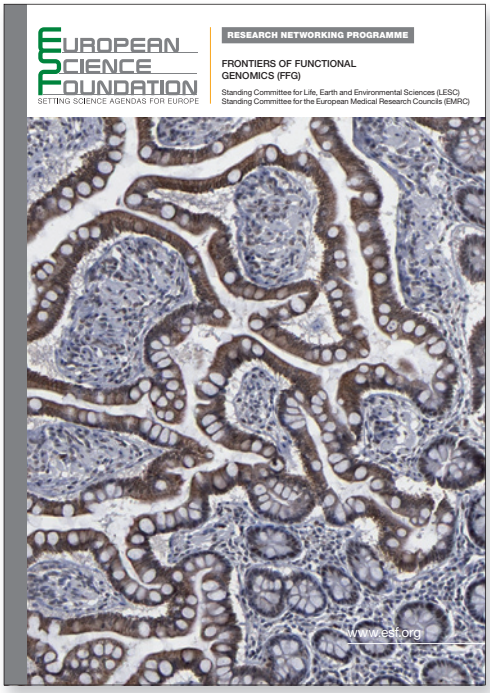
This Forward Look integrated scientists from several EC projects including the RIGHT, SIROCCO, FOSRAK programmes as well as from the RNAQuality EUROCORES programme and the Frontiers for Functional Genomics RNP. The science areas addressed involved careful analyses of future developments in subfields of the RNA world ranging from basic science on RNA quality, bacterial sRNAs and eukaryotic microRNAs, to the pathology of pre-mRNA splicing and the potential of RNA interference for the treatment of a variety of diseases including cancer and infectious diseases.

The scientific report '*RNA World – a new frontier in biomedical research*' was published in September 2010 following approval by the EMRC and LESC standing committees. The report was based on three thematic workshops and a final consensus conference that took place between December 2007 and February 2009. The outcomes from each of the three thematic workshops were discussed during the final consensus conference and led to the identification of the following nine strategic priority areas for RNA research:

1. Construction of an RNA expression atlas
2. Epigenetics
3. RNA in bacterial infections and pathogenesis
4. Alternative RNA splicing
5. Structural and functional characterisation
6. RNA structure and molecular dynamics
7. Visualisation of RNA localisation and transport
8. Development and delivery of RNA therapeutics
9. Application of RNA modifiers in the food industry

The final report can be downloaded at:
http://www.esf.org/publications/forward-looks.html





5.2 Frontiers of Functional Genomics (FFG)

This programme follows the very successful programme Integrated Approaches to Functional Genomics. The FFG programme is supported by science and medical research councils as well as academies in 17 participating European countries and is overseen by a steering committee consisting of a representative from each country.

5. Related ESF Activities

The objectives for the FFG programme are to connect the most promising developments in functional genomics technologies with the expanding concept of systems biology, focusing particularly on applications in biomedicine, as well as the environment and implications for society at large.

The FFG programme areas are:

- Emerging Technologies
  - advanced array technologies
  - nanotechnology: single cell and single molecule analysis
  - RNA interference: gene knockdown for functional genomics
- Bioinformatics and Systems Biology
  - bioinformatic aspects of systems biology
  - computational systems biology
- Functional Genomics and Biomedicine
  - epigenetics, epigenomics and cancer
  - neurogenomics and disease
  - metabolomics
  - pharmacogenomics
  - predictive, preventive and personalised medicine
- Environmental Functional Genomics
  - metagenomics
  - biodegradation by microbial populations
- Functional Genomics and Society
  - public dissemination of functional genomics and disease
  - social welfare, risks and ethics
  - biobanking and populations
  - fostering economic development by technology transfer

The programme promotes interaction between scientists from these research areas through sponsoring of workshops, training courses, conferences and grants for short-term interlaboratory visits.
For further information on this programme, please see: www.esf.org/ffg or http://www.functionalgenomics.org.uk/sections/programme/index.htm

6. Conclusions

The RNAQuality programme has been widely perceived as being a very successful enterprise both by the participants and in the judgement of an international panel of independent assessors. All CRPs demonstrated very high levels of engagement and a genuine exchange of personnel, technology and knowledge. For collaborative efforts such as these to be deemed successful they must demonstrate that the whole, in terms of outcomes, is greater than the sum of the parts and in all three CRPs this seems to be the case and there can be no doubt about the scientific excellence of the majority of the outputs. Success is demonstrated on an initial level by the large number of high quality publications in major international journals that ultimately has to be the main objective measure of success. Many of these publications were achieved by the joint efforts of laboratories within individual CRPs offering clear evidence of synergies that are difficult to achieve without an appropriate research network structure such as EUROCORES. Additionally, other publications resulted from the joint efforts of laboratories from distinct CRPs.

A major strength of the programme's activities that has emerged is one of multidisciplinarity, achieved through the use of a wide range of experimental methodologies and organisms. Such broad approaches cannot be undertaken by individual researchers and require an organised research network strategy.

The key focus on networking activities within the programme offered exciting opportunities for younger scientist to participate in the network through exchange visits. A particular highlight was the meeting in Strasbourg, which was organised by and for young researchers that, as well as providing a great instrument for training was a clear benefit to the whole consortium. In addition a number of international scientific meetings, workshops and summer schools were organised through the RNAQuality programme, perhaps more than can be reasonably expected from a programme of this size. These culminated in spring 2010 with a meeting sponsored jointly with EMBO. Through these efforts the participants have excelled in terms of dissemination and, as judged by external reviewers, the research has acquired visibility throughout the European research community and beyond. These forms of exchange are critical to cementing the success of the funding for future developments but also allowed significant levels of technology transfer that further enhance the future proofing of the funding.

A number of specific scientific highlights are noted below and the review panel acknowledge that a significant number of outputs are still pending, mainly due to the time constraints of the scheme.

1. New, world-leading discoveries related to the structure and function of the exosome.
2. The extension of studies of cryptic and unstable RNAs (PROMPTS) in the nucleus.
3. Connecting RNA interference, RNA degradation and mRNA transport to neuronal cell function.
4. The first identification of phospho-regulation of components of the RISC complex.

More generally, a number of further suggestions are listed below:

Recommendations

1. Continued and increased emphasis on providing access for graduate students and postdocs to advanced technologies.
2. ESF should identify the fields in which Europe is/could be leading the scientific research by bringing together excellent scientists.
3. Projects should not scientifically copy the EU and national funding. They should be unique for innovative and 'out of the box' proposals.
4. As the panel acknowledged that larger CRPs were more productive, a minimum size of four people is recommended which should include at least one PI within the first five years of independent research.
5. ESF should be more pro-active in disseminating information pertaining to Calls and their outcomes, and these efforts should involve the national funding agencies.

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