EUROPEAN SCIENCE FOUNDATION

SETTING SCIENCE AGENDAS FOR EUROPE

FORWARD LOOK

RNA World: a new frontier in biomedical research

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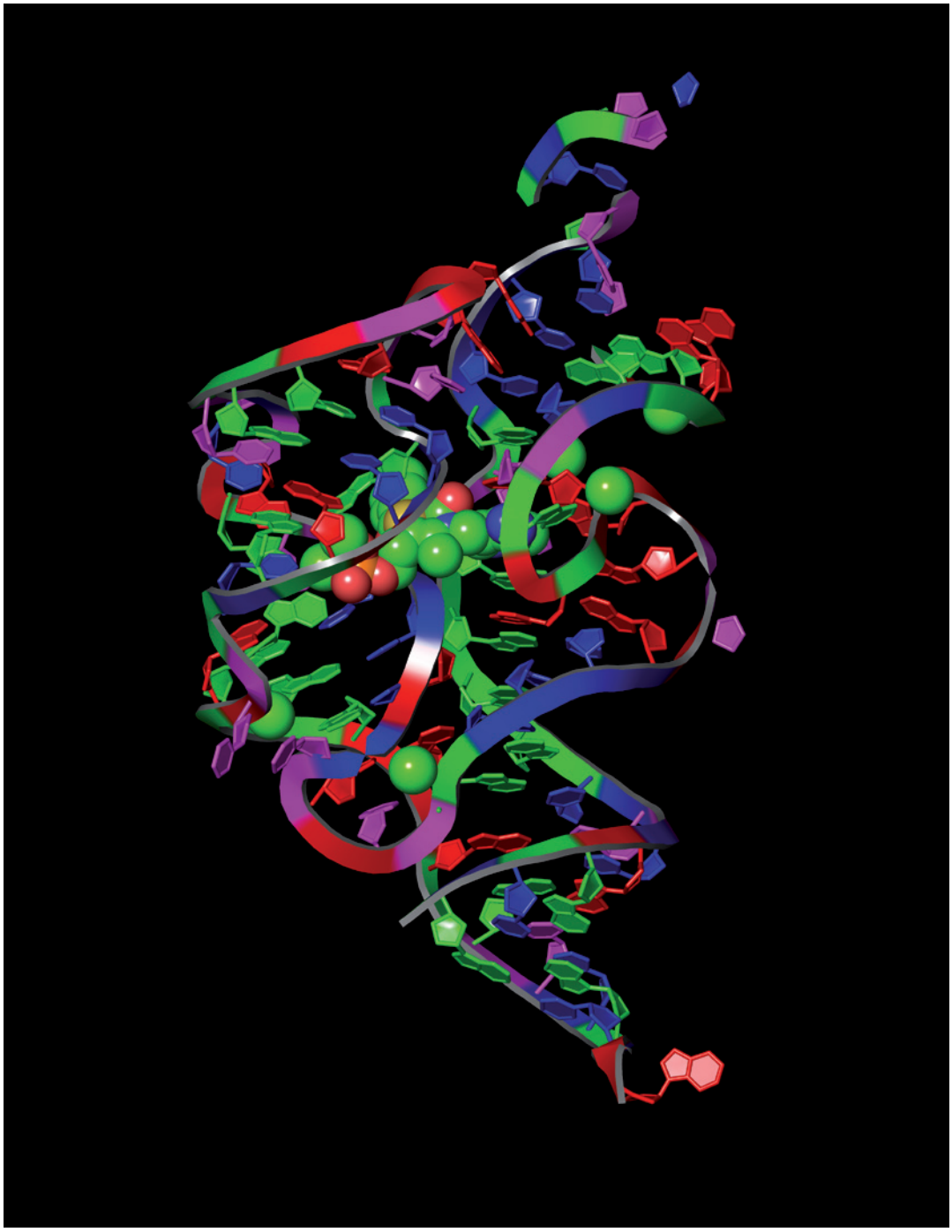


Crystal structure of an E. coli Thi-box riboswitch bound to benfotiamine (Protein Data Bank code: 2hoo; Nucleic Acid Database code: ur0103; Edwards, T.E. and Ferré-D'Amaré, A.R. 2006. Crystal structures of the Thi-box riboswitch bound to thiamine pyrophosphate analogs reveal adaptive RNA-small molecule recognition. Structure **14:** *1459–1468). Image details: RNA: ribbon-plate representation, A-red, U-magenta, G-green, C-blue; Ca ions, benfotiamine: space-filling representation. The image was generated with the Accelrys Discovery Studio Visualizer.*
Image provided by the Jena Library of Biological Macromolecules-JenaLib (www.fli-leibniz.de/IMAGE.html).

Foreword

Ribonucleic Acid (RNA) has been postulated to be the key molecule that led to the origins of life on Earth. This so-called RNA World hypothesis would imply that RNA plays a complex, multi-faceted role in the cellular homeostasis rather than merely serving as a messenger in the transfer of information from DNA to RNA as was believed for nearly 40 years. The discoveries of the last ten years not only brought many unknown functions of RNA to light, findings that were awarded with several Nobel Prizes, but also led to hope and hype that RNA based medicines could bring significant advances to the diagnosis and treatment of human diseases.

The European Medical Research Councils (EMRC) and the Standing Committee for Life, Earth and Environmental Sciences (LESC) of the European Science Foundation (ESF) recognised that the rapidly evolving field of RNA research was relatively underappreciated in Europe. They proposed to conduct a foresight study designed to explore this area and the potential for clinical use of RNA molecules or molecules interfering with RNA processing, to generate awareness of the progress in the field and to foster European research programmes in member countries aiming for stronger European participation in the global context of RNA research. Following approval by the ESF Executive Board, EMRC together with LESC launched the Forward Look activity *RNA World – a new frontier in biomedical research* in 2007. A series of thematic workshops with European leading experts were conducted in 2008. The current status of the field and its forthcoming scientific and funding necessities at the European level, not only for ongoing discoveries but also for biotechnological and pharmaceutical exploitations, were discussed during the workshops and the final consensus conference in 2009.

This report is the outcome of the activities of the ESF Forward Look *RNA World – a new frontier in biomedical research*. It contains a set of specific recommendations dedicated to the needs and requirements of European RNA research. The report has been subjected to quality assurance through peer review by international experts in the field. With the publication of the science policy outlined in this report, ESF aims at facilitating initiatives to be taken by the relevant bodies, including ESF Member Organisations and the European Commission, for actions based on these recommendations. These may involve calls through FP7, other instruments within the EC, calls and special programmes conducted by national funding institutions.

Finally, we would like to warmly thank all of those who were involved in this Forward Look and congratulate them for the important work achieved. Forward Looks are driven by ESF's Member Organisations and by the European research community. These foresight analyses enable Europe's scientific community, in interaction with policy makers, to develop medium to long-term views and analyses of future research developments with the aim of defining research agendas at the national and European level. Our wish is that the recommendations expressed in this report are first listened to and more importantly implemented.

Professor Marja Makarow
ESF Chief Executive

Professor Liselotte Højgaard
EMRC Chair

Executive Summary

Recent years have witnessed an unprecedented gain of knowledge from ribonucleic acid (RNA) research. This caused a paradigm shift from the 40-year-old central dogma that RNA merely serves as a messenger in genetic information transfer to a view that RNA not only plays a role in a multitude of cellular functions, for example in the regulation of gene expression, but also could be the key molecule that led to the origins of life on Earth – the so-called RNA World hypothesis. High expectations and great hype arose from the discovery of RNA interference (RNAi; a mechanism widely employed by eukaryotic cells to inhibit protein production at a post-transcriptional level) which allows gene silencing in experimental settings and has enormous therapeutic potential. RNAi established itself very quickly as a useful molecular biology tool making large-scale functional genomics screens and high-throughput drug target screening possible. There is increasing optimism that RNA-based approaches will bring significant advances to the diagnosis, treatment and prevention of a multitude of human diseases. The exploitation of RNA molecules as diagnostic tools and as therapeutic agents has only begun.

Recognising the high pace of discovery in RNA research and its great potential for therapeutic interventions, the European Medical Research Councils (EMRC) and the Standing Committee for the Life, Earth and Environmental Sciences (LESC) of the European Science Foundation (ESF) decided to conduct a comprehensive foresight study involving European experts from academia and industry to define the status quo of the field, to identify Europe's specific strengths and weaknesses, to establish a 5-10 year outlook for the development of the field and to deliver recommendations designed to foster European research programmes with the aim of ensuring strong European participation in the global context of RNA research. This ESF Forward Look report represents a vision of how this field – both basic RNA research and its translation into clinical practice – should develop. Nine thematic priority areas were identified to address new and promising opportunities for biomedical, biotechnological, pharmaceutical and clinical RNA research.

The Forward Look report *RNA World – a new frontier in biomedical research* is based on three thematic workshops attended by 15 to 25 experts in the field and a consensus conference attended by approximately 100 scientists and policy makers. The meetings took place between December 2007 and February 2009 and were organised by a Scientific Committee which also formed a writing group to assemble this document. The first workshop investigated *Methodologies for RNA Discovery*; the second examined *RNA Interaction Partners* while issues of *RNA Therapeutics* were discussed in the third workshop. The outcomes of the thematic workshops were revisited during the consensus conference which focused on the topics *science, science policy* and *funding*.

Recommendations

Infrastructure, Education and Funding

Europe has only few centres specifically funded to RNA research. We strongly recommend funding of local RNA centres that should be linked to form a Europe-wide *Virtual Institute for RNA Research*. These centres should be multidisciplinary and consist of a critical mass of strong groups working in disciplines such as biology, biochemistry, chemistry, genetics, bioinformatics, biophysics, structural analysis, microbiology, plant sciences and clinical medicine. The centres would be exquisitely suited to train a new generation of young scientists, PhD students and postdoctoral researchers. Dedicated education programmes for RNA research are currently lacking. As in almost all areas of life sciences, a growing demand for qualified scientists can be foreseen especially in bioinformatics. Thus, we recommend financial support for the training of a new generation of bioinformaticians.

The Funding of RNA research in Europe is insufficient and out of proportion with the increasing importance of this rapidly advancing field. A strong impact of RNA research on healthcare can only be achieved with a significant increase of funding dedicated to the investigation of basic principles of RNA function in a variety of model systems, in parallel with focused medically and therapeutically oriented projects. New models for the funding of academic-industrial partnerships should be established to share the risk of developing promising compounds into RNA based medicines.

Scientific priority areas

1. Construction of an RNA expression atlas of living species

- Comprehensive cataloguing of non-coding and coding RNA in cells and tissues, in humans and model organisms in the diseased and normal state
- Defining temporal and spatial expression patterns
- Exploitation of deep sequencing and development of direct RNA sequencing technologies
- Capture and mapping of modified RNA species
- Handling of massive RNA sequence data/infrastructure for bioinformatics
- Computational algorithms to distinguish signal from noise
- Building maps of biomarkers that have potential clinical significance

2. Epigenetics
- Discovery of RNA molecules that program long-lasting gene expression changes
- Characterisation of factors and mechanisms of epigenetic programming

3. RNA in bacterial infections and pathogenesis
- Identification and characterisation of regulatory RNA in infectious bacteria
- Fast infection typing in humans, animals and plants by exploiting small RNA as novel biomarkers
- RNA-based treatment of infection as alternatives to resistance-prone antibiotics
- Treatment of intracellular bacterial pathogens
- RNAi screens for host factors of infection

4. Alternative RNA splicing
- Cataloguing of alternative splicing events in healthy and pathological cells
- Identification and mechanisms of factors that modulate alternative splicing
- RNAi screens to identify regulators of alternative splicing

5. Structural and functional characterisation of RNA-protein complexes
- Identification of stable and transient complexes, and binding sites in RNA and proteins
- Systematic tagging and discovery of partners of RNA-binding proteins
- Three-dimensional structure determination at high resolution

6. RNA structure and molecular dynamics
- High-throughput approaches for RNA structure mapping *in vitro* and in a cellular environment
- Development of new chemistries to asses dynamic RNA structure
- Structural resolution of RNA in complex with proteins or ligands
- Kinetics of RNA folding and association with binding partners
- Defining a language of structure motifs in complex RNA molecules
- Integration of RNA structure data into RNA atlases

7. Visualisation of RNA localisation and transport
- Real-time imaging of RNA at the single-cell, subcellular, sub-tissue and organ levels
- Development of direct RNA detection tags
- Intracellular visualisation of RNA binding to protein partners
- Biocomputational algorithms for kinetic assessment of intracellular RNA structure changes

8. Development and delivery of RNA therapeutics (for humans and animals)
- RNA drugs to silence or correct gene expression with better efficacy
- Liquid phase RNA synthesis to yield sufficient quantities for clinical applications
- New chemistry and vehicles for targeted delivery
- Introduction of RNA drugs into clinical trials

9. Application of RNA modifiers in food industry
- RNA-based biomarkers of non-human diseases (in plants and animals)
- Reprogramming of metabolic processes in microbial organisms to optimise production of food ingredients and new biodegradable materials

Implementation of the recommendations and funding of the scientific priority areas outlined above may require a concerted action of ESF Member Organisations, the European Commission, charities and public-private-partnerships. The European Science Foundation is committed to taking the necessary steps for action based on the recommendations derived from this Forward Look *RNA World – a new frontier in biomedical research*. A first step should involve calls through FP7 and other instruments within the EC as well as calls and special programmes through national funding institutions.

I. Introduction

Ribonucleic acid (RNA) is a class of macromolecules that is found in every living cell. For many years it was believed that RNA was simply a 'messenger', carrying genetic information from DNA to the cell's protein-manufacturing machinery. But in recent years it has become clear that RNA has multiple other roles in the cell, and perhaps most significantly it is now known that RNA is directly involved in the control of gene expression. This discovery has revolutionised our understanding of gene regulation and it holds great promise for significant advances in both basic science and biotechnology and medicine.

Based on research during the 1960s, the Nobel Laureate Francis Crick formulated the 'Central Dogma' of molecular biology, stating that genetic information flows in one direction: from DNA to RNA to protein. In this model, RNA is simply a passive messenger while the proteins are the molecules that do all the jobs in a cell. This original proposal would soon have to be modified, however, as a result of new knowledge. One such modification was introduced after the discovery of the enzyme reverse transcriptase – resulting in a Nobel Prize being awarded to Baltimore, Dulbecco and Temin in 1975 – which allows information in RNA to be 'back-transcribed' into DNA.

Some years later another remarkable aspect to RNA was revealed: that it can behave as a catalyst. This groundbreaking discovery led to a Nobel Prize to be awarded in 1989 to Altman and Cech. Up until then, it was assumed that proteins – enzymes – were responsible for all catalytic work in the cell. The fact that RNA can both carry genetic information and act as a catalyst has led to the suggestion that it could be the key molecule that led to the origins of life on Earth – the so-called RNA World hypothesis.

A third discovery which even further complicated the original simple role of RNA as a messenger of information from DNA to proteins was the discovery of the phenomenon of splicing – which resulted in a Nobel Prize being awarded to Sharp and Roberts in 1993. These researchers showed that the genes of eukaryotes (organisms whose genetic material is contained within a nucleus, which excludes bacteria) contain a mixture of both protein-coding and non-coding structures, called exons and introns, and that the RNA transcript had to be somehow processed before it could be translated into protein: essentially cut up and stitched back together to remove the non-coding regions and put the coding regions in the correct order. Therefore, there was no longer a simple direct 'linear' relationship between the information in DNA and the resulting proteins.

A still more complicated function of RNA was introduced by the later discovery of 'regulated alternative splicing' where different modes of processing the RNA transcript can result in many different forms of proteins from a single gene. Given the central role of RNA and RNA-binding proteins in gene regulation it is not surprising that mutations, insertions or deletions at regulatory sites lead to genetic diseases, as do deregulation or mutation residing within the regulatory proteins or RNAs.

Despite the emerging multiplicity of roles for RNA, most biochemistry textbooks continued to focus on the Central Dogma, that the major role of RNA was to take 'orders' from DNA and pass information on to proteins. However, there was already experimental evidence for a more active role of RNA in controlling gene expression.

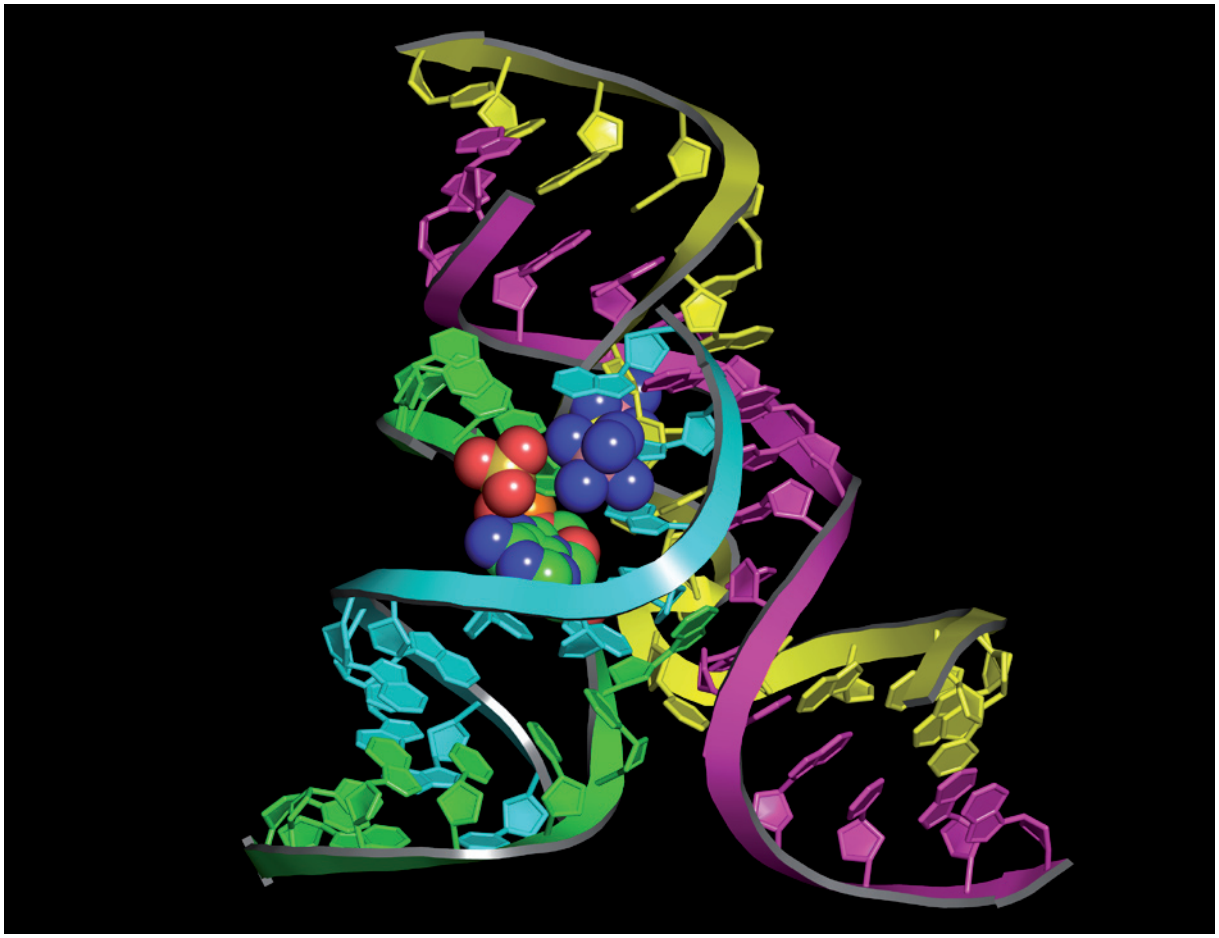
Regulation of gene expression by so-called antisense-RNA molecules had been known since the early 1980s in bacteria. This involves a strand of RNA which does not code for a protein interacting with a complementary strand of RNA that does code for a protein, and thereby preventing the protein from being manufactured. However, for some reason these discoveries had little impact on research in eukaryotic systems. Furthermore, regulation of translation by antisense RNA in the worm C. *elegans* was reported 1993 but somehow this observation was regarded as an oddity of worms.

The breakthrough came with the publication in 1998 by Fire, Mello and co-workers where they described the phenomenon of 'RNA interference' in the worm, where a gene is silenced by the introduction of double-stranded RNA into the cell. The publication led to an explosion of research into RNA and in 2006 Fire and Mello received the Nobel Prize for their discovery.

Fire and Mello showed that RNA interference acts post-transcriptionally – in other words not at the level of DNA before the gene is activated, but after the RNA transcript is formed. In RNA interference, the double-stranded RNA is processed by an enzyme called Dicer to create 'short interfering RNAs', or siRNAs, which are between 21 and 25 nucleotides long. One of the two strands of each fragment of RNA is incorporated into a protein complex called RISC (RNA induced silencing complex) and guides the complex to a messenger RNA (mRNA) which has the complementary sequence to the guide strand. The mRNA is subsequently degraded. RNA interference cannot be explained by simple nucleic acid hybridisation – where complementary sequences of nucleic acids attach to one another – but represents a new phenomenon that requires complicated cell machinery formed from many different gene products. It is a catalytic mechanism with amplification steps – few siRNA molecules in a cell can give very high rates of repression.

RNA interference therefore represents a new regulatory system consisting of a network of enzymatic reactions and molecular signal amplifiers based on double-stranded RNA. Soon after RNA interference was described, in 2001 a number of laboratories reported the discovery of a large class of regulatory 'microRNAs' (miRNAs) in various eukaryotes. miRNAs are produced from endogenous genes and work via a mechanism which overlaps and shares many components with that of RNA interference. Primary RNA transcripts fold into double-stranded 'stem-loop' structures, and are processed via Dicer or Dicer-like enzymes into 21-nucleotide RNAs. These molecules repress the expression of one or more target mRNAs with complementary sequence by an siRNA-like mechanism which brings about the degradation of mRNA or the repression of gene translation. In short, miRNAs are part of a fundamental mechanism for controlling the flow of genetic information in a cell. The human genome codes for around 500 miRNAs and individual miRNAs can each target hundreds of distinct mRNAs. miRNAs are also thought to play a role in diseases such as cancer and cardiovascular disease.

The regulation of gene expression at the level of RNA in mammals is extremely complex, and different cell types rely on non-coding RNAs and RNA-binding proteins (RBPs) expressed in specific cells to modulate gene expression in parallel with cell-type-specific transcriptional regulation. In bacteria, many of these



X-ray structure of a minimal, hinged hairpin ribozyme. (Protein Data Bank code: 2p7f; Torelli, A.T., Krucinska, J., and Wedekind, J.E. 2007. A comparison of vanadate to a 2′-5′ linkage at the active site of a small ribozyme suggests a role for water in transition-state stabilization. RNA **13**: *1052–1070.) Image details: RNA: ribbon-plate representation, substrate strand-yellow, loop A ribozyme strand-green, loop B ribozyme strand-orange, loop B S turn strand-brown. The image was generated with the Accelrys Discovery Studio Visualizer. Image provided by the Jena Library of Biological Macromolecules—JenaLib (www.fli-leibniz.de/IMAGE.html).*

processes are absent; nevertheless, non-coding RNAs and regulation at the mRNA level is also critical. Novel small non-coding RNAs (sRNAs) are being identified in bacteria at a staggering rate and shown to target mRNA or protein and to regulate sophisticated circuits such as stress responses, bacterial virulence or 'group behaviour'. The siRNAs are complemented by long non-coding RNAs which can regulate gene expression through a diversity of mechanisms which at present are still poorly understood. The definition of 'riboswitches' in 2002, metabolite-binding RNA structures residing in the 5' untranslated regions (5'UTR) of bacterial mRNAs, was another unexpected major finding that illustrates the versatility of RNA to bind with high affinity to small molecules. These riboswitches are 'mRNA-effector complexes' which adopt a distinct structure resulting in inhibition or activation of gene expression by interference with transcription or translation.

RNA recognition is also a critical process in innate immunity. DNA and RNA stimulate the mammalian innate immune system by triggering a variety of sensors, including a group of molecules called Toll-like receptors (TLRs). Most DNA and RNA from natural sources contain modified nucleosides. For example a chemical modification termed methylation of particular sequence motifs within DNA blocks signalling via a specific TLR. Similarly, introducing modified nucleosides, such as 2'-*O*-methyl ribonucleosides, into immunostimulatory RNAs can eliminate their immune-activating properties. Understanding these effects has potential importance in clinical applications – in the preparation of vaccines, for example, or in predicting potential side effect for antisense and RNAi therapies.

RNA interference has in a very short time become a new biochemical tool to shut off genes in various organisms to study their function. The great value of this approach was immediately recognised by researchers all over the world and the method of silencing gene expression by siRNA is now widely used. The technique has clear medical applications in combating various diseases, from infection and metabolic diseases to cancer. siRNA-based systems also hold great promise for development into a new therapeutic platform to address novel yet undruggable targets. siRNA-based drugs entered clinical trials as soon as five years after the discovery of RNAi in the worm.

RNA research is now more active than ever and shows great importance and potential. Numerous extraordinary and often entirely unanticipated discoveries have been made in basic research on RNA. These revolutionary discoveries – several of which have resulted in Nobel Prizes – have greatly advanced our understanding of fundamental life processes and have emphasised the key roles of RNA. In addition, many aspects of these studies have a direct impact on medicine and biotechnology.

However, despite these remarkable advances it is important to remember that we are still in the 'discovery phase' and that it may be some years before new technologies based on these discoveries reach the clinic. A good analogy is the development of clinically useful monoclonal antibodies. The first monoclonal antibodies were produced 1975, the Nobel Prize to Köhler and Milstein for the discovery of monoclonal antibodies was presented in 1984, and about ten years later the first clinically useful antibodies against rheumatoid arthritis, non-Hodgkin lymphomas and metastasising breast cancer were produced. Monoclonal antibodies are now showing increasing clinical success. RNA interference was discovered 1998, the Nobel Prize to Fire and Mello came in 2006 and it may be realistic to expect clinically useful drugs based on RNA to start to appear in about ten years from now.

The aim of this Forward Look report is to anticipate scientific developments of broad impact triggered by the recent key discoveries described above. The report will also present guidelines on how to foster a productive research environment in RNA biology by integrating various scientific disciplines and how to harness this new knowledge to develop new and effective treatments for genetic diseases.

II. Current Status of RNA Research and its Translation into Clinical Practice

A Forward Look into Future Developments (as of August 2009)

1. RNA Discovery, Expression and Localisation

1.1. Exploring the unexpected diversity of RNA molecules in living species

Traditionally, the two main roles of RNA had been seen to be in genetic information transfer – as mRNA, and as the genetic material of some viruses – and in protein synthesis – as ribosomal RNA (rRNA) and transfer RNA (tRNA) adaptors. In contrast, the regulatory potential of RNA appeared to be mainly confined to small antisense RNAs that control some specialised biological functions. It turns out, however, that the role of RNA is more complicated, and that there is an unexpected wealth of RNA species in all organisms. In fact, the past decade has seen an explosion in newly discovered types of RNA which do not code for protein – non-coding RNAs – which have a myriad of other important functions in controlling how and when information from DNA is transcribed into mRNA, and then translated into proteins.

Higher organisms, including humans, are now known to produce a variety of non-coding RNAs smaller than 30 nucleotides which are involved in development and disease. They have been shown to be involved in many important biological processes and have been linked to a wide range of diseases, such as cancer and heart disease. Short interfering RNAs (siRNAs) are produced from longer double-stranded segments of RNA and act as a primitive type of defence against pathogens by binding to and causing the destruction of mRNA. MicroRNAs (miRNAs) are found in a wide range of organisms including single cell algae, plants, vertebrates and their associated viruses. Conservative estimates have put the number of miRNAs in humans at around 500, and each one is thought to regulate many mRNAs, with the estimate that most human genes could be regulated by miRNAs independent of their transcription.

A type of RNA called piRNA guards germ cell genomes from the activity of mobile genetic elements, and forms an RNA-based innate immune system that discriminates transposons from endogenous genes and selectively silences the former. Protozoa, an important class of eukaryotic pathogens, generate small RNAs to distinguish “useless” DNA from “useful” DNA in chromosomes during sexual reproduction. It also appears that active transcription of a gene is often associated with the generation of small RNAs in the promoter or transcription termination region; these small RNA species might constitute a signal that is sensed by the cell as a signal to keep a record of genes that are active in a given tissue or at a defined developmental stage. Small RNAs are also used to silence DNA at the chromatin level to induce long-lasting epigenetic programmes.

Similar to eukaryotes, bacteria, including pathogens with high relevance to human, animal and plant health, have over the last decade been found to express many small non-coding RNAs (sRNAs). Although the machinery is different from eukaryotes, the sRNAs also target mRNA or protein to regulate sophisticated circuits such as bacterial virulence or group behaviour. Riboswitches, that is metabolite-binding RNA structures residing in the 5’ untranslated region (5’UTR) of bacterial mRNAs, were another unexpected major discovery illustrating the versatility of RNA for high-affinity small molecule binding, which bears great potential for therapeutic targeting of RNA. Lately, small RNAs associated with the CRISPR loci have emerged as novel and wide-spread RNA-based immunity system against invading DNA elements.

We have also come to appreciate that antisense transcription is a global phenomenon that might have important roles in controlling gene expression after the initial step of transcriptional initiation. The size of antisense transcripts varies among and within organisms. Similar, higher organisms express longer non-coding RNAs of several hundred to a hundred thousand nucleotides in length that act to modulate the activity of proteins or affect the activity state of chromatin by coating chromosomal DNA.

The expression of coding and non-coding genes typically includes a series of nuclear events following transcription from the DNA template. The primary transcript is ‘matured’ by removing non-coding intervening sequences (called introns) by a process known as splicing, and the 3’ end of transcripts is cleaved and polyadenylated. Splicing as well as 3’ end processing may occur in alternative ways, leading to the production of various mRNA isoforms which might encode different non-coding RNA or protein variants. Although the process of alternative splicing has been known for decades, its importance in multiplying information from a defined set of genes is only beginning to be understood.

1.2. RNA expression atlas

Despite the staggering discoveries of new RNA classes in recent years, there is evidence to predict that we have seen only the tip of the iceberg. Even for intensely investigated classes of miRNAs, their definite number in organisms is not settled, with estimates for the number of miRNA genes in humans ranging from 500 to over 1000, depending on the prediction method used. In addition, small RNA discovery was often driven in a few model organisms or cell lines that are not necessarily disease-related.

II. Current Status of RNA Research and its Translation into Clinical Practice

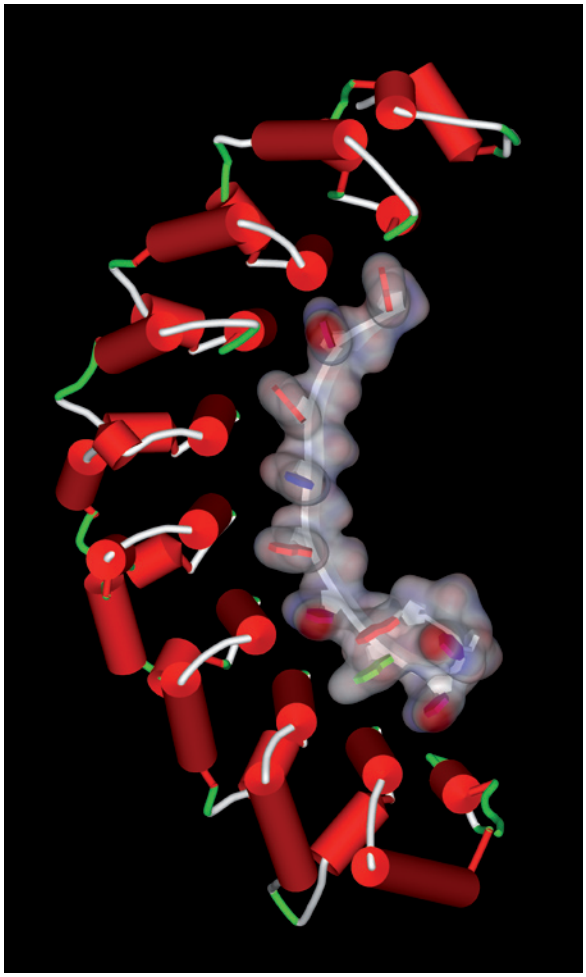
A Forward Look into Future Developments (as of August 2009)

The advent of 'deep sequencing' has like no other recent technology revolutionised the way by which we can discover new RNA molecules and image their expression in living species, from the level of the single cell to that of the entire organism. At present, three technologies (454 pyrosequencing, Solexa and SOLiD) are well established, and new technologies including single molecule sequencing are about to enter the market. Nonetheless, all of these technologies rely on conversion of RNA molecules to cDNA prior to, or at the time of, sequencing, and this introduces potential biases. It is therefore highly desirable to develop technologies that allow us to directly sequence single RNA molecules in a high-throughput fashion.

Deep sequencing will enable the detection of all expressed full-length transcripts and their isoforms from healthy versus diseased material and this will result in the compilation of 'RNA expression atlases'. Such reference maps will result in a better understanding of RNA function in the cell in general, and the identification of regulatory RNAs whose loss of function or malfunction can be targeted for treatment. In addition, given that RNA sequencing is fast, there is great potential to use it for the detection of biomarkers that will direct treatment in situations that require a rapid and/or complex response, for example, infections (sepsis) or cancer.

Beyond general RNA expression profiles for comparative analyses, deep sequencing has the potential to reveal global interaction maps of all RNA molecules with other cellular RNA partners, and especially with cellular proteins. The cellular network of RNA-RNA and RNA-protein interactions is complex, given that, depending on the organism, more than 20,000 protein-coding transcripts and thousands of differentially expressed non-coding RNAs and RNA-binding proteins are known. Deep sequencing can also report the interaction sites of RNAs, to reveal where proteins recognise their target RNA to facilitate post-transcriptional regulation. Reciprocally, the RNA binding partners of cellular proteins should be mapped in a systematic manner. Given that regulation by RNA is highly controlled in a spatial and temporal fashion, RNA atlases are required that resolve RNA expression profiles at a subcellular and sub-tissue level.

Albeit having only four building blocks, RNA molecules undergo complex folding pathways to result in their active structure. The deciphering of the structural language of RNA has been hampered by a paucity of sensitive methods to analyse RNA structures in a cellular environment, and in a high-throughput fashion. Thus, the development of new approaches for RNA structure determination both in a biological context and *in vitro* must be an integral part of the proposed RNA atlases. We also know that



Crystal structure of the Pumilio-homology domain from human Pumilio 1 in complex with Nanos Response Element 2-10 RNA (Protein Data Bank code: 1m8y; RNA: ribbon-plates representation, A-red, U-magenta, G-green, C-blue; protein: ribbon reresentation, helix-red, turn-green). The image was generated with ViewerLite from Accelrys, Inc. Image provided by IMB Jena Image Library of Biological Macromolecules (www.imb-jena.de/IMAGE.html).

RNA molecules can be modified (and edited) to modulate their activity, and therefore any RNA expression atlas must take into account possible modifications – in both the design of the experimental approach and in assigning the functional state of the RNA molecule.

The novel type of RNA atlas will provide genome-wide regulatory maps that need to be established to define the points of interaction, i.e. base-pairing interactions and protein-binding to RNA segments. This should happen on a genome-wide scale in different biological systems for full characterisation of the RNA regulatory

networks. This goal can be accomplished by applying RNA sequencing technology to produce data from all tissues, individuals, model systems, mixed host-pathogen samples, from which we can extrapolate information about the whole organism – in health and disease. This analysis will define interaction maps and will catalogue changes in expression and splice patterns of mRNAs and non-coding RNAs. We know very little about the roles of non-coding RNAs in the interactions and cell-to-cell communication of organisms, for example when humans become infected by viruses or bacteria. Understanding RNA will permit us to modulate the regulatory networks in ways that can treat or prevent disease.

1.3. RNA compartmentalisation

The activity of RNA molecules is highly dependent on where they and their target molecules are located within the cell. The study of RNA localisation has traditionally relied on biochemical fractionation of cells, and these classical approaches need to be further developed and integrated into the building of RNA expression atlases. This will help discover and understand the often large RNA-protein complexes that underlie RNA-based regulation.

Visualisation of RNA molecules in cells is an emerging field that is being fuelled by recent breakthroughs in tracking single RNA molecules in living cells. The current bottleneck is the paucity of *in vivo* labelling technologies that enable direct visualisation of RNA molecules, equivalent to the tagging of proteins with Green Fluorescent Protein (GFP) which allows fluorescent signals to be tracked in real time. Studying RNA localisation in small organisms (most bacterial pathogens) has been a particularly hard task and will require a major effort in the coming years.

The first technologies for RNA tagging exist (aptamers, molecular beacons) but do not yet provide direct and unbiased read-outs to visualise single RNA molecules in complex samples and in real time. Real time imaging methods are required to define kinetics of the expression of RNA molecules in the cell, and the pharmacokinetics of RNA-based drugs once they are in the body. It can also report the non-specific and specific binding and dynamic exchange of RNA with protein complexes to reveal where, when and how RNA enters and leaves the scene at any one time in the concentrated protein soup of the cell. If combined with structural data obtained by *in vitro* studies, RNA imaging will reveal the full picture of splicing and transport mechanisms that underlie targeted gene silencing or activation.

To define the cellular machinery that RNA molecules require for their regulatory activities, genome-wide RNAi or knockout screening approaches will be needed to score perturbations of subcellular arrangements of RNA and protein interaction partners in a particular process, including translational regulation, RNA splicing, RNA editing, and RNAi. Likewise, systematic tagging of all proteins in model organisms is desirable to determine in an unbiased manner which proteins commonly associate with RNA, thereby identifying new building blocks for cellular RNA-protein interactions.

1.4. Annotation by bioinformatics

As part of the genome-wide theme, a major effort is needed for the creation of databases that contain annotation of the full array of coding or non-coding transcripts. Neither the building of RNA atlases by deep sequencing nor the structural analysis of RNA molecules, alone or in complex with interaction partners, will be achievable without a large investment in bioinformatics. Biocomputational analysis is the key to fully capture the degree of gene expression regulation by RNA, to unravel expression and association patterns of RNA with disease and infection, and to evaluate the relative success of RNA drugs upon treatment. Thus, investment in computational biology will enable fully integrated and predictive analyses.

The investment is required at several levels. First, the wealth of data generated by deep sequencing is unprecedented, and is about to exhaust existing computing capacity. Therefore, any of the necessary investment in new sequencing technology has to be matched by an increase in computing hardware. Second, new computational methods are needed that can interpret function, and classify transcripts and their regulation, and can define a language of RNA-mediated regulation. Given the poorly understood diversity of RNA molecules synthesised in living organisms, it is crucial to develop methods to distinguish signal from noise. This concerns computational methods as well as quantitative biology analysis for determining threshold levels of regulators in cells. Third, a new generation of scientists will have to be trained that is able to combine computational analyses with an understanding of the specific biological properties of RNA molecules.

Regarding biomedical applications, that is the research into disease and infection with pathogens (bacteria, virus), and the development of RNA-based diagnostic tools and therapeutics, links with clinicians will have to be fostered. The procurement and sharing of large numbers of clinical samples for RNA analysis will require strong bioinformatics in order to explain common genetic diseases or susceptibility to infections by alterations of sequences and RNA-based regulation.

II. Current Status of RNA Research and its Translation into Clinical Practice

A Forward Look into Future Developments (as of August 2009)

2. RNA Biology and Function

Understanding the many biological roles and the molecular mechanisms employed by small and large RNAs is of utmost importance. This is not only because we need a deep understanding to satisfy our curiosity about fundamental life processes *per se*, but also because development of diagnostic tools and intervention strategies in disease treatment and prevention demand a full comprehension of what is happening inside cells. This means that basic questions have to be asked and addressed before or in parallel with attempts to use this knowledge in medical and biotechnological applications. This calls for a broad range of approaches, from genetics to establish RNA function in whole organisms, to cell biology to reveal those changes that are mediated on the cellular level, to sophisticated biochemistry to obtain a detailed molecular understanding of the RNAs themselves and their interaction partners (many proteins, other RNAs, low molecular weight ligands, and possibly chromatin/DNA/chromatin-associated proteins).

In spite of a staggering pace of progress in the expanding RNA field, many central questions remain unanswered. In particular, the complexity of prokaryotic and even more eukaryotic regulatory systems in which RNAs have major roles needs to be addressed. RNAs rarely or never act alone; they rely on helpers, often proteins. Many well-known molecular machines such as the ribosome and the spliceosome are complex and complicated RNPs (ribonucleoprotein particles). Regulation by miRNAs, for instance, works through the mediation of protein effectors. In this case, the small RNA can be viewed as the specificity determinant that guides the effector proteins to the site of action, the target sequence typically in the 3'-UTR of a metazoan mRNA or the coding region of a plant mRNA. In general if we want to understand how RNA regulates numerous processes within the cell, it is essential to understand how RNA and proteins interact at the molecular level. Thus, questions of the composition of RNA-protein and RNA-RNA complexes are critical, as is our understanding of what determines the rate and specificity of the interactions. Here the field of chemical biology could play a key role.

Therefore, we need to find and characterise the interaction partners of RNAs and address this both by single in-depth case studies and through large-scale systems biology approaches. The latter calls for an ambitious development of new methods for ribonucleoproteomics. Both of these approaches have their strengths and limitations, but both are needed. Another crucial issue is that of structures. Very few RNA structures are known in atomic detail, and even fewer RNP structures. Why should we care about RNA structure? Pioneering studies have revealed that RNA – unlike DNA – forms intramolecular three-dimensional structures that are not simply built up from Watson-Crick base pairs (as is the case with DNA), but use numerous 'non-canonical' contacts to make up the complex tertiary fold architecture that is functionally significant. By the same token, regulatory interactions between RNA molecules or RNA with proteins have generally been assessed only qualitatively. Current knowledge suggests that the kinetic aspects of interactions are pivotal in biological systems in which RNAs have key roles as regulator. Very little has been done to investigate these quantitative and kinetic constraints even though these have deep implications for medical applications. To understand fully how RNAs will behave in cells – in medical treatment for example or using RNA as a way to silence a gene in an organism – specificity and rate considerations are crucial. At this point, we do not even understand how 'off-target' aspects are avoided in natural regulatory systems. Clearly, biophysical studies will play a key role in answering these questions.

2.1. Small non-coding RNAs

A topic that has soared over the last five to ten years deals with small RNAs. These molecules which are often classified according to their biogenesis, their biochemically recognisable features, or their general functional roles, are found ubiquitously in all living organisms. The vast majority, but not all, work by antisense mechanisms, implying that base-pairing to target nucleic acids is the key step that provides recognition of the molecule that is to be regulated. Initially, antisense RNAs were discovered as regulators of gene expression in bacterial plasmids. Today, we know that bacterial chromosomes encode numerous members of a heterogeneous class of small RNAs (denoted sRNAs) that generally inhibit yet can also activate the expression of proteins in the context of stress responses and/or in virulence traits in pathogens.

Correspondingly, in eukaryotes, numerous functionally or biochemically distinguishable classes of sRNAs have received unprecedented attention. A common denominator for many of these is a size range of 21-25 nucleotides, which is a direct consequence of their being generated from double-stranded RNAs by an RNAse III-type enzyme called Dicer. One such class of RNAs, uncovered in the aftermath of the discovery of RNA interference (RNAi), is called siRNA (small interfering RNA). They are processed from externally introduced or internally produced long double-stranded RNAs and enter a pathway that results in the targeted degradation of mRNAs that share sequence homology. These RNAs do not act alone but require assembly into an effector complex, RISC, that comprises a number of associated

proteins. RISC composition differs between organisms and probably cell type, but a necessary key player is a member of the Ago (argonaute) family of RNA binding proteins (RBPs). Ago proteins most often have "Slicer" activity; that is they cleave the target RNA guided by the base-pairing of the specific complementary siRNA.

The miRNAs (microRNAs) are derived by processing from endogenous precursor transcripts that have double-stranded character. miRNAs are regulators of many cellular processes in a wide range of multicellular organisms. The mechanisms by which miRNAs work are still somewhat controversial, but translational inhibition and induced mRNA degradation have convincing support. It is already clear that miRNAs are in many cases strongly associated with disease and development. Overexpression or the absence of an miRNA has been associated with cancer, cardiovascular syndromes and many other diseases.

A new fascinating class of sRNAs, generated in a Dicer-independent pathway, are the piRNAs (PIWI-associated RNAs). PIWI proteins are a subclass of the argonautes and also possess Slicer activity. The piRNAs are almost exclusively expressed in the germline and play an important role in the defence against rampant genetic elements (such as transposons) that threaten genome integrity. Defects in PIWI/piRNA generation strongly influence germ line development.

The above is by no means an exhaustive list of such short RNAs, and emphasis has been given to post-transcriptionally acting RNAs. A plethora of partly related variants have been discovered in various organisms, plants being here an important source of information. It is clear that we have only seen the beginning of the complexity of sRNAs in eukaryotes, and it can be expected that many functionally relevant "classes" have so far escaped detection due perhaps to heterogeneity in their properties. This is particularly applicable to recent findings of siRNA-like RNAs that affect transcription and/or promoter activity.

2.2. Long non-coding RNAs

Even though a small number of mammalian long non-coding RNAs have been known for many years, it is clear from sequencing studies and transcriptional profiling that many more are present. The function of only a few of these long non-coding RNAs has been addressed. These long non-coding RNAs appear to be nuclear rather than cytoplasmic. What are long non-coding RNAs doing and how do they act? The best-known examples are found associated with epigenetic phenomena such as imprinting in mammals. The association of RNA with epigenetic modifications such as DNA methylation (mostly in plants) and histone modifications (many systems, best studied in fission yeast) strongly suggests a role for RNA in chromatin remodelling.

Another role appears to be in 'dosage compensation' in the case of mammalian X chromosomes. Inactivation of one of the X chromosomes in each cell follows a stochastic initiation process which is dependent on a non-coding RNA. This RNA – in a process whose molecular details remain obscure – coats the chromosome and recruits protein factors that promote formation and maintenance of the silent state. In a similar fashion, maternally or paternally imprinted loci on autosomes are silenced on only one of the two chromosomal copies, in a manner that is specific to the parent of origin. Almost invariably, specific non-coding RNAs are expressed from the silenced locus and are required for silencing. Additional non-coding RNAs function in transcriptional control of genes, for example by interaction with a transcription factor to modulate expression of downstream genes.

Many long RNAs have been observed by large scale transcriptional profiling or a technique called EST-cloning. Often, they are described as antisense RNAs. Today, our understanding of long non-coding RNAs remains limited. This is in part due to the difficulty of discovering them and establishing their functional significance (excluding transcriptional noise). A second reason lies in the complexity and the overall low tractability of such systems, which have severely hampered detailed mechanistic investigations.

Finally, it is worth emphasising that long non-coding RNAs overall may work in ways that are totally different from those characteristic of small RNAs. It is also likely that the level of control may be distinct. Small RNAs are excellent in targeting effector complexes to RNAs, favouring post-transcriptional control, whereas very long non-coding RNA may possibly be better at introducing large scale chromatin changes, that is, epigenetic effects. Since epigenetics is a topic of tremendous current interest, and the involvement of RNA in this phenomenon is now clearly established, new tools and concepts for the study of RNA's role in this regard are urgently needed and should be developed.

2.3. RNA interaction partners

RNAs of the classes mentioned above do not act on their own. The si- and miRNAs are part of RISC and miRNP complexes; spliceosomal RNAs reside in the huge spliceosome which consists of numerous proteins; the ribosome is an RNA-protein machine for the template synthesis of proteins, to mention just a few. Most other RNAs also have specific protein partners. A general feature is that most of these RNPs are in dynamically changing states – the composition of the particles determines the state of activity or promotes localisation when

appropriate. In particular, many complexes are transient and involve only weak binding; the most stable structures are not necessarily the most biologically significant. Techniques need to be established to 'freeze' or stabilise transient associations so that they can be identified and quantified, and 'snapshots' of these various structures can be taken. Recent studies in bacteria result in similar conclusions. The simplest but best-studied case involves Hfq (an RNA binding "helper" protein) which is found in complex with most regulatory sRNAs and which can additionally recruit RNA-degrading enzymes called ribonucleases (RNAses) for downstream effects. However, nearly half of the known bacteria – including important pathogens – do possess Hfq, suggesting that other general RNA-binding proteins and associated classes of non-coding RNAs are to be discovered.

Given the importance of RNPs in all aspects of life, it is troublesome that our information about their composition, dynamics of assembly and disassembly, and functional steps, is so limited. It is of high relevance to chart the RNA-binding partners in biologically relevant complexes, and to identify their roles in cellular processes. This should be done broadly using high throughput approaches but also by running state-of-the-art biochemistry programmes on selected RNPs – as has been done, for example, with the spliceosome.

Apart from biochemistry, genetics can be a powerful tool in revealing the function of potentially important proteins in RNPs. One important avenue is to make better use of bioinformatics. There is a potential to mine data to track the co-evolution of RNA and specific proteins to provide evidence of plausible associations. With richer and more detailed information it will be possible to use systems biology approaches to chart all the interactions that are important for regulation.

RNAs also need to interact with RNAs. This is already obvious from the description of bacterial antisense RNAs, miRNAs and siRNAs. In all cases, specific target sequence recognition must take place. We know that antisense-target complementarities can be very short. How then is efficient specific target recognition achieved, and mispairing to undesired targets ('off-target' effects) avoided? For RNA-RNA binding processes, including proteins that might aid or prevent this, there is a paucity of knowledge regarding the kinetics of interaction, the modulation of interactions by proteins, the impact of the structures of the interaction partners on binding, and the overall properties of the cellular environment that may affect the outcome of binding.

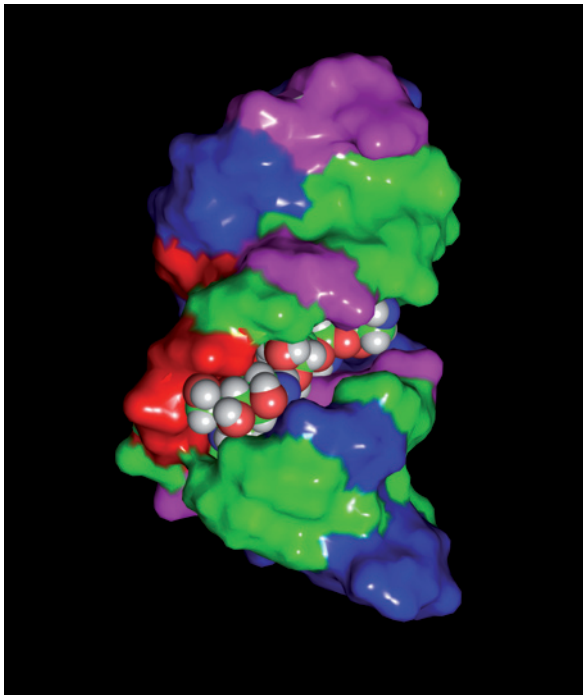
RNA-RNA interactions also occur in ways that do not involve "simple" base-pairing but rather complex interactions of structural elements such as RNA tetraloop receptors. Again, this type of interaction has been found in the context of larger RNAs (rRNA, ribozymes) but their importance for general regulatory function is at this point largely speculative.

Interactions between many low-molecular-weight ligands and structured RNAs have already received much attention. Such interactions, mediating regulation of the activity of RNAs, are referred to as riboswitches. They are abundantly found in bacteria where they regulate a multitude of genes. What is not clear is whether they are also present in eukaryotes, and if so whether they could account for biological properties that are yet unexplained.

For all interactions between RNA and its partners, a deep understanding of biological significance requires analyses of association and dissociation on a relevant time scale, either *in vitro* or in the cell. It is likely that standard *in vitro* techniques will not be sufficient to capture the full picture of what is happening in the cell. Events at this scale are very fast and new methods in kinetics will be needed to produce a meaningful understanding of the system. Key questions in RNA regulation revolve around where and when within the cell specific events occur. One promising approach is time-resolved intracellular imaging. This involves *in vivo* fluorescent tagging of RNA. There is much room for innovation in this area, with respect to the development of new fluorescent tags that do not interfere with the fundamental properties of the RNA, methods that improve time resolution on single molecules, and "multiplexing" so that many different molecules can be observed in different colours simultaneously. Such approaches can provide important information on the dynamics and kinetics of RNA transport and association with proteins.

2.4. Regulation of RNA

It has been a longstanding dogma that in normal development and in disease, mRNAs constitute a central point of regulation. One important aspect is related to splicing. Many genes can give rise to alternative mRNAs, generated by a process denoted 'alternative splicing'. Here, activation or inactivation of specific splice sites, most often controlled by tissue- or cell-specific protein factors, leads to changes in splice site selection and therefore a changed distribution of mRNA isoforms. All being well, this in turn results in appropriate protein output to serve the needs of the cell. Aberrant splicing therefore can result in abnormal protein composition and consequently can cause disease. At the same time, mRNA with Premature Termination Codons (PTC) resulting from erroneous alternative splicing or genome rearrangement can be rapidly detected by the nonsense-mediated decay (NMD) pathways and targeted for degradation. A better understanding of the details of splice site selection



Solution structure of a eukaryotic decoding region A-site oligonucleotide bound to paromomycin (Protein Data Bank code: 1fyp; Lynch, S.R. and Puglisi, J.D. 2001. Structural origins of aminoglycoside specificity for prokaryotic ribosomes. J. Mol. Biol. ***306:*** *1037–1058). Image details: model 1 shown; RNA: ribbon-plate representation, red-brown; paromomycin: space-filling representation, atom-specific coloring. The image was generated with the Accelrys Discovery Studio Visualizer.*
Image provided by the Jena Library of Biological Macromolecules (JenaLib; www.fli-leibniz.de/IMAGE.html).

and regulated RNA decay is a key objective that must be further pursued.

Mature mRNAs are also the targets of regulation by many RBPs, often but not always guided by miRNAs. The perplexing multitude of interactions that has been recognised to date, and its extrapolation, suggest that one could view particularly the often unusually long and phylogenetically conserved 3' UTRs of human and other animal mRNAs as the platform at which regulation occurs. This regulation is modular and highly dynamic: many RNPs can act simultaneously with their specific sites, sometimes by cooperation, sometimes counteracting each other. It is also clear that the outcome of a regulatory interaction can vary. miRNAs often promote translational inhibition but recent reports indicate that activation can occur. Recruitment of certain classes of proteins to miRNPs can also result in mRNA destabilisation. Finally, localisation plays an important role for the outcome. Translationally repressed mRNAs generally are found in particular cellular granules called P-bodies from which they may even be retrieved upon cell stress.

Beside the direct mRNA regulation mechanisms described above, additional ways of controlling RNA levels in the cell and consequently impacting on gene expression have emerged as active scientific fields. RNA surveillance mechanisms accurately identify RNA molecules with defects in processing, folding, or assembly with proteins acting to rapidly degrade them, avoiding inadequate translation. In addition these mechanisms also ensure the processing of pre-RNAs into mature and functional molecules. Surprisingly, RNAs produced by several different types of RNA polymerases and presenting no structural or functional similarities are nevertheless all controlled by the same surveillance mechanisms. Although the reasons of degradation are broadly the same across the different classes of RNAs, the substrates do not show any common structural features. Those controlled degradation and processing pathways are operated by multi-enzyme complexes such as the exosome and its cofactor in the eukaryotes and the degradosome in prokaryotes. Those complexes are currently under detailed scrutiny for their multiplicity of function and to decipher the key mechanisms for identifying and targeting correct RNAs and RNA-protein complexes.

For ribosomal RNAs, pre-rRNA maturation is closely controlled by the exosome and its cofactor. In the case of ribosomes stalled on mRNA, the 'no-go decay' pathway is activated and the mRNA degraded. Additionally, under starvation conditions, mature ribosomes can undergo entire phagocytosis and under various stresses rRNA can be cleaved; in all cases, those mechanisms result in rapid translation inhibition. Similarly tRNA anticodon loops can be cleaved under developmental regulation or in response to oxidative stresses to repress translation.

One major challenge is to derive a comprehensive picture of regulatory interactions in their dynamic state, correlated with function. This again should be done in a two-pronged approach. High throughput methods can yield information on proteins/RNAs bound to specific mRNAs in different physiological states, in different cell types, during treatment with chemicals, and so on. In-depth studies on selected model mRNAs will yield information about the key aspects of regulation in molecular detail. The choice of model system is also important. Clearly, different models have contributed to different aspects of our knowledge to date. This is exemplified by the key contributions of plant research to our understanding of RNA silencing. It is impossible to predict where significant breakthroughs will come, and thus model systems should represent a spectrum of organisms.

II. Current Status of RNA Research and its Translation into Clinical Practice

A Forward Look into Future Developments (as of August 2009)

3. RNA and Disease

RNA as the transcript of DNA is the platform for translation into proteins. Thus interfering with the translation of a protein considered causative for a disease by blocking or degrading its mRNA is one approach to combat disease. Unsurprisingly this strategy was the first to be adopted. However, mRNAs contain not only sections (exons) coding for a protein but also non-coding sections (introns). In the process of mRNA maturation the introns are spliced out. Splicing can be variable so that certain exons are removed, producing a shorter but still active protein. Sometimes essential exons are spliced out resulting in an inactive protein, and a large number of human diseases are caused by such alternative 'unfaithful' splicing. Examples include Duchenne muscular dystrophy and spinal muscular atrophy (SMA). Thus, repairing faulty splicing, for example, by delivering suitable oligonucleotides, could help treat these diseases.

The cellular endogenous RNA surveillance mechanisms to target mutations after transcription and thereby prevent the production of truncated or erroneous proteins could be another strategy. For example, nonsense-mediated decay (NMD) has been associated with druggable human diseases, and compounds that correct nonsense mutations via NMD have been identified and brought to the level of clinical trial, as in the case of nm cystic fibrosis treatment. Nonsense mutations are known to cause a significant proportion of several other important human diseases, such as haemophilia and Becker muscular dystrophy.

Correct translation is a prerequisite for obtaining a particular protein. In addition, the appropriate timing and the correct amount of protein production is essential for normal physiology. Regulation of these factors is at least in part exerted by miRNAs through the repression of translation. Overproduction of miRNAs will result in the loss of a protein (e.g. a tumour suppressor) or at least in reduced production which in turn can be the cause of a disease and this is for example observed for many cancers. On the other hand, certain cancers are associated with a lack of miRNAs as it leads to production of proteins which normally are repressed (e.g. anti-apoptotic proteins). As a consequence, modulating the amount of miRNAs is a strategy to combat such diseases. Similar strategies are in principle applicable to combat viral infections – especially given that some mammalian and plant viruses encode miRNAs, interfere with the function of host miRNAs, or encode factors that fight host defence. Finally, nucleic acids also hold the promise to target bacterial and protozoan/fungal infections.

4. Diagnostics and Therapies

4.1. RNA as a diagnostic tool

Is it feasible to obtain RNA profiles from serum and use these to provide useful and meaningful information? The issue is contentious. There are reports that the presence of a tumour can be detected by searching for miRNAs in blood or stool samples using the technique of single molecule PCR, which can pinpoint a single mutated molecule in one million. However, whether a miRNA profile of a blood sample can be used as a way to diagnose disease is not clear. People are also looking at miRNA profiles from tumour samples to obtain information about the tumour type.

Deep sequencing and high-throughput screening will continue to develop over the next five to ten years, and this is likely to provide the tools for making detailed miRNA profiles of tissues. Precisely how the profiling of miRNAs and of pathogenic RNA will provide information about a disease and how that will impact on treatment remains to be resolved.

4.2. Therapeutics

The potential for RNA as a therapeutic has become much more evident recently as we learn about the various roles RNA plays in cellular processes. A drug strategy based on RNA has the particular attraction that genes responsible for a disease can be targeted at their coding sequences on the mRNA, thereby preventing the production of the protein that causes the disease. This, together with the accuracy of base-pairing, could lead to novel, specifically targeted drugs. Oligonucleotides that target RNA are more specific than small molecular drugs, which usually interact with the protein of choice but frequently with others as well, causing undesirable side effects.

Assessing how efficacious a therapeutic RNA molecule is *in vivo* requires the measurement of outputs 'as close to the action' as possible: measuring transcriptome and proteome outputs is crucial. Similarly, because the biology of the system is complex, it is necessary to have more than one end-point. It is important to run tight controls and the issue of species specificity is also crucial. Targets must be well validated. How RNA drugs behave *in vivo* is another central issue.

One of the key questions relating to pharmaceutical safety is whether there is anything special or unique about a given molecule; in this regard innate immunity to RNA is an issue that will require close scrutiny (see 4.3 Immunology). It seems likely that most toxicity from RNA therapies will not derive from RNA *per se* but rather in its

conjunction with the delivery vehicle. The side-effects of chemical modification of RNA to increase its efficacy also need to be assessed for safety implications.

Off-target effects need to be considered carefully. These can be modelled *in silico*, but also need to be addressed experimentally, although this kind of study might lie in the remit of the drug developer rather than the academic scientist. Given the nascent state of the field, however, inputs from academic scientists will nevertheless be required.

As research pushes RNA technology towards clinical use, methods need to be found to manufacture appropriate quantities of these molecules. Solid phase synthesis is well established and can cope with kilogram quantities, but for larger-scale manufacture it is likely that liquid phase techniques will need to be developed.

As a general point, it is worth noting that in terms of oligonucleotide-based therapeutic development Europe is lagging well behind the US. At present clinical trials in Europe represent only 10% of the total, with the remainder in US. It is important for Europe to take up the challenges that new scientific discoveries present and to become globally competitive in the field of RNA therapeutics.

4.2.1 Antisense-based gene silencing

The use of small interfering RNA (siRNAs) as a gene silencing mechanism has been highly successful in a relatively short period compared with classical antisense approaches. One big outstanding issue is the interaction of exogenous RNAs with toll-like receptors, proteins that play an important role in the innate immune system. The consequence of interactions of siRNAs with these receptors and their effect on the immune response is important and should be studied closely (see below). Delivery of siRNA remains a critical issue. In contrast to the single-stranded antisense oligonucleotides, AONs, the double-stranded siRNAs are not easily taken up by cells (see below). However, once taken up they are much more efficient than the AONs and thus represent significant potential as therapeutics. There are other aspects of basic research around siRNA and its activity that can improve its potential as a therapeutic technology, for example devising ways to increase the lifetime of siRNA in the RISC. For short siRNA hairpins there is a need to make these more specific to improve vectors.

Translation arrest is an approach that uses AONs to block the ribosome binding site of messengers in order to prevent the onset of translation. These oligonucleotides do not activate enzymatic cleavage of mRNA and thus a large amount of oligonucleotide material is needed and it is necessary to apply it continuously as it has to be applied stochiometrically. Translational arrest remains a popular tool in developmental biology, however, and there is activity within the antiviral area.

One advantage that AONs have over siRNA as a gene silencing approach is that AONs can generally be delivered to the cell more efficiently as they apparently use endogenous cell-uptake machinery; while siRNA can be far more specific to a particular cell type, it is more difficult to get it into the cell because of its double-stranded nature.

One problem with AONs is that they tend to end up primarily in the liver, but less so in other organs setting a challenge to target other organs and tissues. Here, chemistry can help as specific ligands can direct the AON more precisely, particularly towards tumours. Various conjugation chemistries are being investigated to improve targeted delivery for high potency. The antisense approach requires recognition by the enzyme RNaseH, which limits the 'chemical space' available to work within. This could be the reason that this approach is less efficient intracellularly than the siRNA methodology. That being said, approximately 20 AONs are in clinical trial.

Our understanding of the biology of miRNA is presently far from complete but it is widely agreed that this is an area of enormous potential which should be pursued vigorously in an effort to answer the many outstanding biological questions. In the context of therapeutics it is important to realise that many miRNAs are associated with certain diseases, so far primarily recognised in various cancers and tumours, where miRNAs are overexpressed in some but under-represented in others. Additionally, as miRNAs are involved in regulation of gene expression it is likely that they also play an important role in other conditions, such as heart disease. As a therapeutic strategy, therefore, miRNA could be applied exogenously for diseases resulting from a lack of the molecule, or in cases where there is an overexpression of miRNA, an oligonucleotide complementary to the miRNA could be administered, thereby inhibiting the miRNA (a so-called antagomir). The first examples of such applications are beginning to be researched, but the work remains in its infancy. As each miRNA has a large number of targets, the decision about which miRNA should be chosen is critical.

Target validation is a key goal; it is necessary to understand distribution and role of miRNAs and other non-coding RNAs in disease aetiology. This is a potentially fruitful area for academic and industrial collaboration which will require high-throughput genome-wide screening. New animal models of diseases will be needed to pursue this aim. There is also a need for studies aiming to understand how different chemically

modified oligonucleotides are taken up, with these results feeding back into improved chemistry for targeting miRNA.

4.2.2 Splice correction

Splice correction involves preventing incorrect splicing by AONs or using functional tethers to activate exon splicing. This is an area that has great potential in a large number of diseases. The first proof of principle of splice correction was shown in 2001, and the first clinic trial, in Duchenne muscular dystrophy (DMD), started in 2006; the field is advancing rapidly. The ability to target sites in the exon enables a very high degree of specificity.

We are beginning to understand how to design AONs for splice correction, and there are now efforts to discover if the rules that apply for DMD also apply to other exons. It is thought that around 15 per cent of mutations result in splice aberrations, and that splice aberrations are the main cause for a large number of diseases.

As long as the RNA sequence can be targeted specifically, it is possible to make many more modifications than with RNase H-dependent systems. Work on RNase H-dependent mechanisms began much earlier because it was thought that much higher efficacy would be possible. However, the biology of this has been slow to develop because larger amounts of material than anticipated need to be delivered.

Importantly, splice correction results in a gain of function, in contrast to many genetic approaches which rely on silencing. While it is usually necessary to completely silence a rogue gene, often only a relatively modest gain of genetic function is needed to produce a measurable and therapeutically useful phenotypic response; complete restoration of the gene is not needed. In addition, splice correction is a much easier approach from the medical point of view as it is less likely than other approaches to trigger immune regulatory systems.

4.2.3 Aptamers

Aptamers are oligonucleotide sequences that can selectively and specifically bind to other molecules. An aptamer that binds to a particular protein, for example, can interfere with that protein's activity. In this way aptamers have some similarities to antibodies. One issue relating to aptamers is whether they should be developed in direct competition to antibodies or whether it would be better to develop them against different set of targets, taking advantage of their unique properties such as their small size. A useful property of aptamers is that they can be switched off by a complementary oligonucleotide which abolishes the affinity to the protein, thus enabling a protein to be inhibited only temporarily. Aptamers offer additional advantages over antibodies: as the selection is a biochemical process, it is possible to target any protein including toxins and non-immunogenic targets, and the selections can be done under conditions that reflect those of final use (which is particularly important for components of biosensors or nano-devices).

The construction of aptamers is proving a challenge – they are difficult to make. There has been relatively little effort at incorporating new chemistries, but various groups are now beginning to take this up. There are also moves to develop new ways to select aptamers for functionality. Historically they have been selected for binding, resulting in inhibition of the protein targeted, but there are signs that aptamers could be selected for other functions such as helping to transport molecules into specific cell types, or delivery of molecules across specific biological barriers. Thus, cell-type-specific aptamers could deliver therapeutics such as AONs or siRNAs. Generally, although the first aptamer (against wet age-related macular degeneration) was approved as a drug in 2005, and additional first clinical trials are under way, this field of research is at a relatively early stage in relation to the development of therapies.

4.3. Immunology

Nucleic acids have a number of interesting immunobiological properties. CpG oligonucleotides, for example, are one of the most powerful adjuvants for B-cell activation. The properties of RNA are immunologically diverse. Significantly, it is known that the immune response to nucleic acids is very different in mice than it is in man due to different expression patterns of receptor proteins. This is important when considering data derived from rodent studies in the development of RNA-based silencing technologies: RNA is much more active in humans than in mice.

In general oligonucleotides can give rise to stimulation or silencing of the immune response. There are situations in which these might or might not be desirable. The chemistry of the oligonucleotides plays an important and as yet poorly understood role in this differentiation. The field requires further exploration.

It is also important to understand the influence of the transfection agent on the immune response and to dissect this out from the response to the RNA payload. There need to be new assay systems that can determine immunostimulation with and without carriers.

4.4. RNA delivery

One key issue for RNA delivery for therapeutic applications is that it is desirable to achieve not only cell-type-specific targeting, but also escape from the

endosome (the membrane-enclosed compartment in the cell into which external particles are ingested) once the RNA has been delivered. A modular approach could be feasible, with different modules incorporated into the delivery system: a targeting module and an escape module, and a module to increase circulation time to avoid excretion, for example. There needs to be greater understanding of the biology of endosome formation and maturation. More work is needed on the precise mechanisms of delivery, which would allow a more rational approach to the design of delivery systems for therapeutic applications. In addition, delivery of RNA or derivatives thereof to small sized organisms such as bacterial pathogens is yet to be fully developed, and will require major efforts.

Carrier systems based upon liposomes – small hollow vesicles made from lipids – have many attractive features. They protect the payload from degradation, improve the lifetime through the serum, have good uptake properties, and so on. However, current systems can be complicated and techniques need to be devised to make them simpler, to reduce their toxicity and to enable a tissue or cell-type specific targeting. People are investigating the use of polymers that show similar properties. In particular negatively charged materials, such as RNA, seem to require some sort of encapsulation – it is difficult to bestow the necessary properties on the compound by attaching ligands. Considerable bioengineering research is currently underway to investigate the development of particles. The problem here is that these are often difficult to reproduce – something that is not the case with chemical conjugates, for example. Some self-assembled polymer systems are relatively cheap and reproducible. It is important to avoid complicated nanoparticles – simpler systems are needed.

A number of ligands, such as folate, are showing promise for targeting tumours, and the selection of ligands for cell surface markers is a growth area. There is currently no systematic effort for screening for abundant surface marker proteins and this could be a valuable exercise.

It is difficult to make general assumptions about targeting and uptake of molecules because these are highly dependent on the tissue or cell type. However, it is imperative to have a clear understanding of whether the molecule is actively functioning in the cytoplasm once it has been delivered, and new model systems are needed to assess this. This question will have to be addressed for different cell types of therapeutic interest and for different targeting approaches, as alternative intracellular trafficking pathways may be utilised depending on cell type and internalisation pathway.

Also, there are many different viral vector systems for delivering RNA payloads – the choice of vector depends on the cell or tissue type that is being targeted and on the expertise of the particular lab. While viral systems have been shown to be effective in the delivery of RNA machinery such as mRNA cassettes or short hairpins, there needs to be more effort to optimise platforms. For example, high transfection efficiency is needed for RNA-mediated gene silencing. Non-invasive routes to delivery could be a fruitful and potentially highly rewarding area of research. Possibilities include oral, inhalation and transdermal routes.

At this stage of research, cost need not be an overriding issue. The main quest is for innovations that yield simple, reproducible systems that do the job.

4.5. Academic-industrial partnerships

Beyond research, however, many aspects in this field are inherently expensive – like the development of animal models and pre-clinical work such as toxicology and pharmacology – and academia is unlikely to be able to bear the entire cost. Thus academic-industrial partnerships could help to carry the burden. A big problem is the issue of disclosure – academics need to publish and this is sometimes not compatible with the way that industry proceeds.

One answer to this problem might be to consider a dedicated publicly funded pre-clinical study facility that would possess the necessary infrastructure and resources to take promising new compounds to the pre-clinical phase. Good manufacturing practice (GMP) facilities for researchers investigating new compounds for cancer therapeutics are essential. There are some new models of public funding to stimulate a more rapid translation of scientific research into the clinic.

III. Recommendations

The starting point of our recommendations is that funding for RNA research in Europe, in contrast to the situation in the US, is substantially insufficient and out of proportion with the increasing importance of this rapidly advancing field. A significant increase in funding should support investigations of basic principles of RNA function in a variety of model systems, in parallel with focused medically and therapeutically oriented projects, in order to make a strong impact on healthcare. It is important to bear in mind that many important medical advances have arisen from research that initially had little immediate connection with medicine.

Scientific priority areas

1. Construction of an RNA expression atlas of living species

- Comprehensive cataloguing of non-coding and coding RNA in cells and tissues, in humans and model organisms in the diseased and normal state
- Defining temporal and spatial expression patterns
- Exploitation of deep sequencing and development of direct RNA sequencing technologies
- Capture and mapping of modified RNA species
- Handling of massive RNA sequence data/infrastructure for bioinformatics
- Computational algorithms to distinguish signal from noise
- Building maps of biomarkers that have potential clinical significance

2. Epigenetics

- Discovery of RNA molecules that program long-lasting gene expression changes
- Characterisation of factors and mechanisms of epigenetic programming

3. RNA in bacterial infections and pathogenesis

- Identification and characterisation of regulatory RNA in infectious bacteria
- Fast infection typing in humans, animals and plants by exploiting small RNA as novel biomarkers
- RNA-based treatment of infection as alternatives to resistance-prone antibiotics
- Treatment of intracellular bacterial pathogens
- RNAi screens for host factors of infection

4. Alternative RNA splicing

- Cataloguing of alternative splicing events in healthy and pathological cells
- Identification and mechanisms of factors that modulate alternative splicing
- RNAi screens to identify regulators of alternative splicing

5. Structural and functional characterisation of RNA-protein complexes

- Identification of stable and transient complexes, and binding sites in RNA and proteins
- Systematic tagging and discovery of partners of RNA-binding proteins
- Three-dimensional structure determination at high resolution

6. RNA structure and molecular dynamics

- High-throughput approaches for RNA structure mapping *in vitro* and in a cellular environment
- Development of new chemistries to asses dynamic RNA structure
- Structural resolution of RNA in complex with proteins or ligands
- Kinetics of RNA folding and association with binding partners
- Defining a language of structure motifs in complex RNA molecules
- Integration of RNA structure data into RNA atlases

7. Visualisation of RNA localisation and transport

- Real-time imaging of RNA at the single-cell, subcellular, sub-tissue and organ levels
- Development of direct RNA detection tags
- Intracellular visualisation of RNA binding to protein partners
- Biocomputational algorithms for kinetic assessment of intracellular RNA structure changes

8. Development and delivery of RNA therapeutics (for humans and animals)

- RNA drugs to silence or correct gene expression with better efficacy
- Liquid phase RNA synthesis to yield sufficient quantities for clinical applications
- New chemistry and vehicles for targeted delivery
- Introduction of RNA drugs into clinical trials

9. Application of RNA modifiers in food industry

- RNA-based biomarkers of non-human diseases (in plants and animals)
- Reprogramming of metabolic processes in microbial organisms to optimise production of food ingredients and new biodegradable materials

Structure of activities

Europe has only few centres dedicated to RNA research. To address this problem we strongly recommend the funding of local RNA centres. Such centres should be linked together as a Europe-wide 'virtual institute'.

These centres should be multidisciplinary and have a critical mass of strong groups working in disciplines such as biology, biochemistry, chemistry, genetics, bioinformatics, biophysics, structural analysis, microbiology, plant sciences and clinical medicine.

Such centres would be exquisitely suited for promoting superior training of a generation of young scientists, PhD students and postdoctoral researchers. Dedicated education programmes for RNA research are currently lacking.

A particular area where expertise is lacking and where an increasing demand in the future can be foreseen is, as in almost all other areas of life science, bioinformatics. Therefore, we recommend the training of a new generation of bioinformaticians.

Funding

New models for public funding of infrastructure and resources for promising compounds to be used in the clinic should be developed. The financial burden for taking basic compounds and developing them into drugs should be shared by academic-industrial partnerships.

IV. Committee Members

Management Committee (MC)

Chair
- Professor Lars Thelander, Institute for Medical Biochemistry and Biophysics, Umea, Sweden

Co-Chairs
- Professor Jörg Vogel, Max Planck Institute for Infection Biology, Berlin, Germany
- Dr Annick Harel-Bellan, Institut André Lwoff, CNRS, Villejuif, France

Other Members
- Dr Edvard Beem, EMRC rapporteur, The Netherlands Organisation for Health Research and Development (ZonMw), The Hague, Netherlands
- Professor Jan Motlik, LESC rapporteur, Institute of Animal Physiology and Genetics, Libechov, Czech Republic
- Professor Marja Makarow, ESF, Strasbourg, France
- Professor Liselotte Højgaard, EMRC, France and University of Copenhagen and DTU, Copenhagen, Denmark
- Professor Eero Vasar, EMRC Rapporteur (as substitute to E. Beem at the Final Conference), Department of Physiology, University of Tartu, Estonia

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- Professor E. Gerhart H. Wagner, Uppsala University, Sweden
- Professor Fritz Eckstein, Max Planck Institute for Experimental Medicine, Germany
- Mr Simon Hadlington, Science Writer, York, UK

Scientific Committee (SC)

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- Professor Bengt Nordén, Physical Chemistry, CTH, Chalmers University of Technology, Göteborg, Sweden
- Dr Thomas Tuschl, Laboratory of RNA Molecular Biology, Howard Hughes Medical Institute, Rockefeller University, New York USA
- Dr Hans-Peter Vornlocher, Roche Kulmbach GmpbH, Kulmbach, Germany
- Professor Gerhart Wagner, Department of Cell & Molecular Biology, Uppsala University, Sweden

V. Appendices

Appendix 1

Methodology

The Forward Look 'RNA World – a new frontier in biomedical research' initiative is led by members of national funding and research performing organisations and managed by the LESC and EMRC; EMRC heading the management. The methodology used for the workshops was approved by the Management Committee.

The topic for a Forward Look on 'RNA World – a new frontier in biomedical research' was proposed by the EMRC and LESC Standing committees and written by Professor Jörg Vogel (Max Planck Institute for Infection Biology, Berlin, DE) and Dr Annick Harel-Bellan (Institut André Lwoff, CNRS, Villejuif, FR). The proposal was approved by the ESF Executive Board.

In a launch meeting organised in Strasbourg on 14 March 2007, Professor Lars Thelander (Department of Medical Biochemistry and Biophysics, Umea University, SE), Dr Annick Harel-Bellan and Professor Jörg Vogel agreed to respectively chair and co-chair this activity. On 24-25 May 2007, a preparatory meeting was organised in Sigtuna (SE) to discuss and approve:

- the organisational structure
- the methodology
- the activities time line
- the thematic properties
- the goal and ambitions
- the communication

The Management Committee is composed of the chair and two co-chairs of the Forward Look, the ESF Chief Executive, the EMRC Chair, Dr Edvard Beem (The Netherlands Organisation for Health Research and Development, The Hague, NL) as EMRC rapporteur, and Professor Jan Motlik (Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, CZ) as LESC rapporteur (Figure 1).

A Scientific Committee was formed to lead the analysis of the three strategic themes, with the aim to shape solutions and recommendations. This committee is listed in Chapter IV. It contains the chairs and co-chairs of the three workshops, as follows:

- Workshop 1 '*Methodologies for RNA Discovery'* chaired by Dr Thomas Tuschl (Laboratory of RNA Molecular Biology, Rockefeller University, New York, US) and Professor Jörg Vogel
- Workshop 2 '*RNA Interaction Partners*' chaired by Dr Annick Harel-Bellan and Professor Gerhart Wagner (Department of Cell & Molecular Biology, Uppsala University, Uppsala, SE)
- Workshop 3 '*RNA Therapeutics*' chaired by Professor Fritz Eckstein (Max-Planck Institute for Experimental Medicine, Göttingen, DE), Dr Hans-Peter Vornlocher (Roche Kulmbach GmbH, DE) and Professor Jørgen Kjems (Department of Molecular Biology, University of Aarhus, DK)

Professor Bengt Nordén (Physical Chemistry, CTH, Chalmers University of Technology, Göteborg, SE) and Professor Eero Vasar (Department of Physiology, University of Tartu, ES) also took part of the Scientific Committee activities. The three workshops (Figure 2) and the consensus conference took place from 17 December

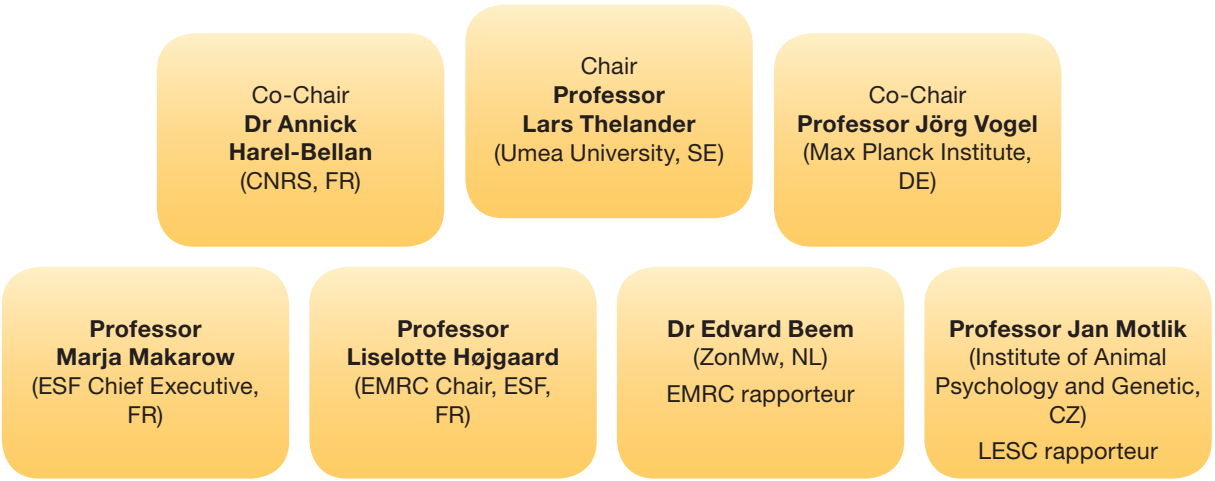

Figure 1. Members of the Management Committee

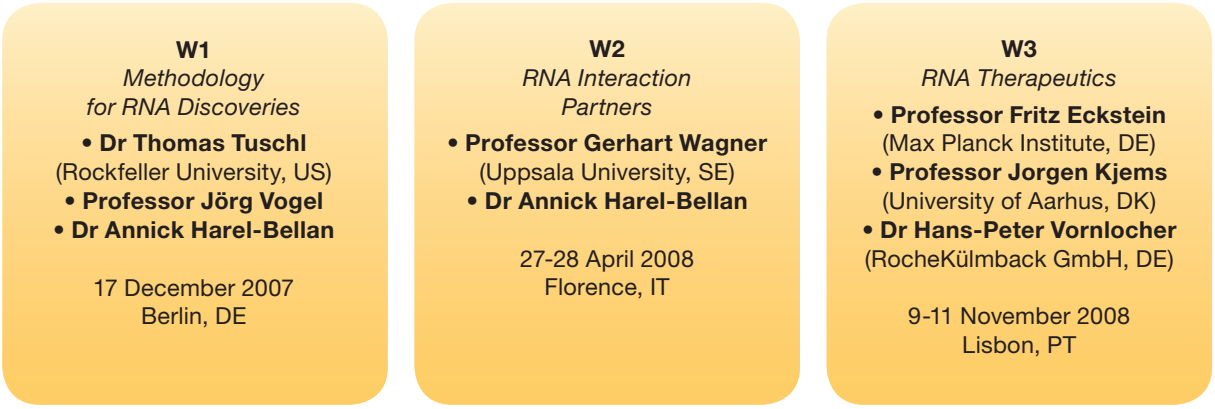

Figure 2. Workshops chaired by the members of the Scientific Committee

2007 to 23 February 2009. The participants[1] for each were invited based on their high level expertise in the field of RNA research.

In each workshop the problems and needs of RNA in biomedical research for the respective theme were identified and discussed. Then recommendations to overcome these problems and needs were formulated and agreed upon through a collaborative problem solving process.

The recommendations were subsequently discussed at the consensus conference held in Granada, Spain on 22-23 February 2009, hosted by Professor Alfredo Berzal-Herranz (Instituto de Parasitología y Biomedicina "López-Neyra", CSIC, Granada, SP) and attended by 102 participants representing key areas in the field. Nobel laureate Professor Thomas H. Cech (Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Colorado at Boulder, US) participated as a keynote speaker. Following the consensus conference, a scientific report was written and nine scientific priority areas were formulated to sharpen the strategy of the RNA research in Europe.

The Forward Look report will be publicised and disseminated widely among all interested stakeholders.

1. (see chapter 'Committee members' and Appendices 2 and 3)

Appendix 2

Workshop Participants

Workshop I. Methodologies for RNA Discovery

17 December 2007, Berlin, Germany

Chair

- Dr Thomas Tuschl, Laboratory of RNA Molecular Biology, Howard Hughes Medical Institute, Rockefeller University, New York USA
- Professor Jörg Vogel, Max Planck Institute for Infection Biology, Berlin, Germany

Participants

- Professor David Baulcombe, The Sainsbury Laboratory, John Innes Centre, Norwich, United Kingdom
- Professor Edward K.L. Chan, University of Florida, Gainesville FL, United States
- Dr Markus Droege, Roche Diagnostics GmbH, Roche Applied Science, Penzberg, Germany
- Professor Witold Filipowicz, Friedrich Miescher Institut for Biomedical Research, Basel, Switzerland
- Professor Lars Thelander, Institute for Medical Biochemistry and Biophysics, Umea, Sweden
- Dr Annick Harel-Bellan, Institut André Lwoff, CNRS, Villejuif, France
- Dr René F. Ketting, Hubrecht Institute for Developmental Biology, Utrecht, Netherlands
- Professor Michael Kiebler, Neuronal Cell Biology, Center for Brain Research, Medical University of Vienna, Vienna, Austria
- Dr Yann Legros, Illumina Ltd, Essex, United Kingdom
- Professor Reinhard Lührmann, Abt. Zelluläre Biochemie, Max Planck Institut für Biophysikalische Chemie, Göttingen, Germany
- Professor Fritz Melchers, Max Planck Institute for Infection Biology, Berlin, Germany
- Professor Thomas Meyer, Max-Planck-Institute for Infection Biology, Berlin, Germany
- Dr Nonia Pariente, EMBO Reports, Heidelberg, Germany
- Professor Nikolaus Rajewsky, Max-Delbrück-Center for Molecular Medicine, AG Systembiologie, Berlin-Buch, Germany
- Dr Pascale Romby, CNRS – ARN, Architecture and Reactivity of RNA, IBMC, Université Louis Pasteur, Strasbourg, France
- Professor Peter F. Stadler, Faculty of Mathematics and Computer Science, University of Leipzig, Leipzig, Germany
- Dr Roland Wicki, Director Alliance Management, Next Generation Sequencing, Molecular Cell Biology, Applied Biosystems, Foster City CA, United States
- Dr Mihaela Zavolan, Biozentrum, Division of Bioinformatics, Universität Basel, Basel, Switzerland
- Dr Jan Motlik, LESC rapporteur, Institute of Animal Physiology and Genetics, Libechov, Czech Republic
- Professor Fritz Eckstein, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany
- Professor Jørgen Kjems, Department of Molecular Biology, Faculty of Natural Science, University of Aarhus, Denmark
- Professor Bengt Nordén, Physical Chemistry, CTH, Chalmers University of Technology, Göteborg, Sweden
- Dr Hans-Peter Vornlocher, Roche Kulmbach GmpbH, Kulmbach, Germany
- Professor Gerhart Wagner, Department of Cell & Molecular Biology, Uppsala University, Sweden

Workshop II. Interaction Partners

27-28 April 2008, Florence, Italy

Chairs

- Dr Annick Harel-Bellan, Institut André Lwoff, CNRS, Villejuif, France
- Professor Gerhart Wagner, Department of Cell & Molecular Biology, Uppsala University, Uppsala, Sweden

Participants

- Dr Denise Barlow, CeMM Centre for Molecular Medicine, Vienna Biocenter, Max F. Perutz Laboratories, Austrian Academy of Science, Vienna, Austria
- Dr Jan Christensen, Department of Biology, Faculty Natural Sciences, University of Copenhagen, Copenhagen, Denmark
- Dr Elena Conti, Structural Cell Biology Department, Max Planck Institute of Biochemistry, Martinsried, Germany
- Professor Michael Famulok, LIMS Institute, Institut für Organische Chemie und Biochemie, Universität Bonn, Bonn, Germany
- Professor Witold Filipowicz, Department of Epigenetics, Friedrich Miescher Institut for Biomedical Research, Basel, Switzerland
- Professor David Lilley, CR-UK Nucleic Acid Structure Research Group, MSI/WTB complex, University of Dundee, Dundee, United Kingdom
- Professor Reinhard Lührmann, Department of Cellular Biochemistry, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
- Dr Marjori Matzke, Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna, Austria
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- Dr Edvard Beem, EMRC rapporteur, The Netherlands Organisation for Health Research and Development (ZonMw), The Hague, Netherlands
- Dr Jan Motlik, LESC rapporteur, Institute of Animal Physiology and Genetics, Libechov, Czech Republic

Workshop III. RNA Therapeutics

9-11 November 2008, Lisbon, Portugal

Chairs
- Professor Jørgen Kjems, Department of Molecular Biology, Faculty of Natural Science, University of Aarhus, Aarhus C, Denmark
- Professor Fritz Eckstein, Max-Planck-Institute for Experimental Medicine, Göttingen, Germany
- Dr Hans-Peter Vornlocher, Roche Külmbach GmbH, Kulmbach, Germany

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- Dr Alfredo Berzal-Herranz, Instituto de Parasitología y Biomedicina «López-Neyra», Parque Tecnológico de Ciencias de la Salud, CSIC, Granada, Spain
- Dr Michael Gait, Laboratory of Molecular Biology, Medical Research Council, Cambridge, United Kingdom
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- Dr Olaf Heidenreich, Medical Sciences, Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom
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- Mrs Julie Clayton, Science Writer – Editor, Bristol, United Kingdom

Coordination
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Appendix 3

Participants of the Consensus Conference

22-23 February 2009, Instituto de Parasitología y Biomedicina “López-Neyra”, CSIC, Granada, Spain

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- Professor Jörg Vogel, Max Planck Institute for Infection Biology, Berlin, Germany
- Dr Annick Harel-Bellan, Institut André Lwoff, CNRS, Villejuif, France

Host
- Dr Alfredo Berzal-Herranz, Instituto de Parasitología y Biomedicina «López-Neyra», Parque Tecnológico de Ciencias de la Salud (CSIC), Granada, Spain

Speakers
- Professor René Bernards, Molecular Carcinogenesis, Netherlands Cancer Institute, Amsterdam, Netherlands
- Professor Thomas H. Cech, Howard Hughes Medical Institute, Chevy Chase MD, United States
- Professor Witold Filipowicz, Department of Epigenetics, Friedrich Miescher Institut for Biomedical Research, Basel, Switzerland
- Dr David V. Morrissey, siRNA Technology, NIBR Biologics Center, Novartis Institutes for BioMedical Research, Inc., Cambridge MA, United States
- Dr Peter Mouritzen, Department of Product Development, Exiqon A/S, Vedbaek, Denmark
- Professor Reinhard Lührmann, Abt. Zelluläre Biochemie, Max Planck Institut für Biophysikalische Chemie, Göttingen, Germany
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- Professor Markus Stoffel, Laboratory for Metabolic Disease, Institute of Molecular Systems Biology, Zürich, Switzerland
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Consensus Conference location: Instituto de Parasitología y Biomedicina « Lopez-Neyra » (CSIC), Granada, Spain

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Appendix 3

Participants of the Consensus Conference

22-23 February 2009, Instituto de Parasitología y Biomedicina "López-Neyra", CSIC, Granada, Spain

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- Ms Belén Martínez, affiliation as above
- Ms Soledad Marton Garcia, affiliation as above
- Ms Marta Montes, affiliation as above
- Mr José Antonio Reyes, affiliation as above
- Mrs Ana Margarita Rodriguez Hernandez, affiliation as above
- Dr Cristina Romero Lopez, affiliation as above
- Mrs Noemí Sánchez, affiliation as above
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- Dr Thomas Bruhn, ESF-EMRC
- Dr Arja Kallio, ESF-LESC
- Ms Blanche Facchini, ESF-EMRC

- Mr Simon Hadlington, Science Writer, York, United Kingdom

Appendix 4

RNA Initiatives funded by the European Commission

- REGULATORY GENOMICS – Advanced genomics instruments, technology and methods for determination of transcription factor binding specificities; applications for identification of genes predisposing to colorectal cancer (Life sciences, genomics and biotechnology for health)
  Jussi Taipale
- MOLTOOLS – Advanced molecular tools for array-based analyses of genomes, transcriptomes, proteomes, and cells (Life sciences, genomics and biotechnology for health)
  Ulf Landegren
- VIRAPT – Antiviral Aptamers for Treatment of HIV-1 Infection (Life sciences, genomics and biotechnology for health)
  Dorothee Von Laer
- CALLIMIR - Biological role of microRNAs in the DLK1 GTL2 imprinted domain. (Life sciences, genomics and biotechnology for health)
  Carole Charlier
- HIDDEN HIV CHALLENGE – Challenging the hidden HIV: understanding the block on transcriptional reactivation to eradicate infection (Life sciences, genomics and biotechnology for health)
  Monsef Benkirane
- VIZIER – Comparative Structural Genomics of Viral Enzymes Involved in Replication (Life sciences, genomics and biotechnology for health)
  Christian Cambillau
- RNABIO – Computational approaches to non-coding RNAs (Life sciences, genomics and biotechnology for health)
  Eric Westhof
- EURO-PHARMACO-GENE – Design of targeted Gene Pharmaceutics using self-assembling functional entities. (Life sciences, genomics and biotechnology for health)
  C. I. Edvard Smith
- HIVSTOP – Development of an effective RNA interference-based anti-HIV-1 therapy using an SV40-derived gene transfer vector (Life sciences, genomics and biotechnology for health)
  Peter de Haan
- AGLAEA – Development of novel animal models of glutamatergic central nervous system disorders using *in vivo* siRNA and transgenic approaches (Life sciences, genomics and biotechnology for health)
  Mark Epping-Jordan
- THE EPIGENOME – Epigenetic plasticity of the genome (Life sciences, genomics and biotechnology for health)
  Thomas Jenuwein
- EUREXPRESS – EURExpress, a European consortium to generate a web-based gene expression atlas by RNA in situ hybridization (Life sciences, genomics and biotechnology for health)
  Andrea Ballabio
- EUTRACC – European Transcriptome, Regulome and Cellular Commitment Consortium (Life sciences, genomics and biotchnology for health)
  Frank Grosveld
- EXCELLENT-HIT – Exploiting Cellular Export of Nuclear Transcripts as HIV Innovative Therapy (Life sciences, genomics and biotechnology for health)
  Myriam Witvrouw
- FOSRAK – Function of small RNAs across kingdoms (Life sciences, genomics and biotechnology for health)
  Martin Tabler
- FSG-V-RNA – Functional and Structural Genomics of Viral RNA (Life sciences, genomics and biotechnology for health)
  Sybren Wijmenga
- CARDIOGENICS – Identification of genetic roots of coronary artery disease by combining stepwise genome wide association studies with transcriptomic and functional genomic investigation of relevant genetic variants (Life sciences, genomics and biotechnology for health)
  Heribert Schunkert
- BLOODOMICS – Identification of risk genes for atherothrombosis in coronary artery disease by transcriptome and proteome analysis and high throughput exon resequencing (Life sciences, genomics and biotechnology for health)
  Nicholas Andrew Watkins
- DROP-TOP – Integration of DNA, RNA and protein markers in a tool for the prognosis and diagnosis of human disease (Life sciences, genomics and biotechnology for health)
  Gorka Ochoa
- BACRNAS – Non-coding RNAs in bacterial pathogenicity (Life sciences, genomics and biotechnology for health)
  Renée Schroeder
- RIBOREG – Novel roles of non-coding RNAs in differentiation and disease (Life sciences, genomics and biotechnology for health)
  Martin Crespi
- RIGHT – RNA Interference Technology as Human Therapeutic Tool (Life sciences, genomics and biotechnology for health)
  Thomas F. Meyer

Appendix 4

RNA Initiatives funded by the European Commission

- SIROCCO – Silencing RNAs: organisers and coordinators of complexity in eukaryotic organisms (Life sciences, genomics and biotechnology for health)
  David Baulcombe
- SLIC – SLIC-Biosensors in Molecular Diagnostics: Nanotechnology for the Analysis of species-specific Microbial Transcripts (Life sciences, genomics and biotechnology for health)
  Solomzi Makohliso
- RIBOSYS – Systems Biology of RNA Metabolism in Yeast (Life sciences, genomics and biotechnology for health)
  Jean Beggs
- ATD – The Alternate Transcript Diversity Project (Life sciences, genomics and biotechnology for health)
  Daniel Gautheret
- TRANS-REG – Transcription complex dynamics controlling specific gene expression programmes (Life sciences, genomics and biotechnology for health)
  Iannis Talianidis

Appendix 5

Selected places with RNA research in Europe

- **Centre for Comparative Genomics, Uppsala, SE**
  www.ucg.uu.se/GWagner.html
- **Centre for mRNP Biogenesis and Metabolism, Aarhus University, DK**
  www.mrnp.dk
- **Centre for Genomic Regulation, Barcelona, ES**
  www.crg.es
- **The Research Institute of Molecular Pathology, Vienna, AT**
  www.imp.ac.at
- **Department of Biochemistry, Cambridge University, UK**
  www.bioc.cam.ac.uk
- **Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK**
  www2.mrc-lmb.cam.ac.uk
- **Institut de Biologie Moléculaire et Cellulaire, Strasbourg, FR**
  www-ibmc.u-strasbg.fr
- **Institut de Biologie Moléculaire des Plantes, Strasbourg, FR**
  ibmp.u-strasbg.fr
- **The European Molecular Biology Laboratory, Heidelberg, DE**
  www.embl.de
- **Max Planck Institute for Biophysical Chemistry, Göttingen, DE**
  www.mpibpc.mpg.de/english/research
- **Gene Center Munich, DE**
  www.lmb.uni-muenchen.de
- **Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, DE**
  www.mpi-cbg.de
- **Max Delbrück Center for Molecular Medicine, Berlin, DE**
  www.mdc-berlin.de

Appendix 6

Selected companies involved in RNA Research and Development

- **Alnylam Pharmaceuticals**
- **Anadys Pharmaceuticals**
- **Altogen Biosystems**
- **Ambion (a division of Applied Biosystems, Inc.)**
- **ArmaGen Technologies, Inc**
- **BD Biosciences**
- **Benitec**
- **Bionomics**
- **BioSpring**
- **Calando Pharmaceuticals**
- **Celera Genomics**
- **Cenix BioScience**
- **Ceptyr**
- **Dharmacon**
- **Genlantis**
- **Ingenium Pharmaceuticals**
- **Integrated DNA Technologies, Inc.**
- **Invitrogen**
- **InvivoGen**
- **Isis Pharmaceuticals**
- **Microsynth AG**
- **Nucleonics Inc**
- **Pfizer Inc**
- **RNAi Co. Ltd**
- **Roche Kulmbach GmbH**
- **Sigma-Aldrich**
- **Sirna Therapeutics, Inc.**
- **SIRION-Biotech**
- **Thermo Fisher Scientific**
- **QIAGEN Sample & Assay Technologies**
- **esiRNA (Endoribonuclease-prepared siRNA)**
- **MISSION esiRNA, Sigma-Aldrich**

Appendix 7

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