European Cooperation in the field of Scientific and Technical Research - COST -

Secretariat

Brussels, 14 December 2009

COST 259/09

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action BM0906: Regulatory RNAs in bacterial pathogenicity: new targets for alternative therapies.

Delegations will find attached the Memorandum of Understanding for COST Action BM0906 as approved by the COST Committee of Senior Officials (CSO) at its 176th meeting on 1 December 2009.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action BM0906
REGULATORY RNAs IN BACTERIAL PATHOGENICITY: NEW TARGETS FOR ALTERNATIVE THERAPIES.

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 270/07 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The main objective of the Action is the pooling of knowledge and coordination at the European level of research in the field of infectious diseases. The understanding of RNA-mediated regulatory networks in pathogenic bacteria requires concerted studies in different organisms including Archaea and yeast. Data from different organisms should be made available and comparable through the usage of compatible techniques and approaches. Finally, strategies used by pathogens to adapt to their hosts are to be deciphered with the aim to identify novel targets for therapeutic intervention.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 52 million in 2009 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter V of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

Bacterial infections continue to be a serious health problem worldwide and research to understand and combat bacterial infections remains indispensable and needs considerable further efforts. Alternative novel approaches are needed to address this important concern. In the past years, a very high number of regulatory RNAs have been discovered. Many of these control bacterial pathogenicity and the adaptation of bacteria to environmental changes and to their hosts. However, these studies have only been performed in a few model organisms and are still in an initial state. It is now urgent to widen the scope of the present research to include all major human pathogens focusing on common mechanisms between pathogens and identifying pathogen-specific targets. This Action connects European research groups from different disciplines working on regulatory RNAs in a large number of human pathogens. The aim of this Action is to bring these experts together allowing synergy between the different approaches to a common goal: to increase basic knowledge on the regulation of bacterial pathogenicity on a genomic scale in order to identify novel targets to develop novel therapies. This Action will boost a novel fast expanding research area by especially promoting young investigators.

Keywords: infectious diseases, bacteria, non-coding RNAs, regulatory RNAs, regulatory networks, host-pathogen interactions

B. BACKGROUND

B.1 General background

Definition of the Research Topic:

Infectious diseases are the second-ranking cause of death worldwide. Yet, despite increasing incidence of bacterial resistance to existing drugs, antibiotics development and discovery in the pharmaceutical industry is declining. Many of the current drugs used to treat bacterial infections target just a few classes of enzymes, briefly those involved in synthesis of proteins, nucleic acids, cell wall or folate. Our objective is to concert European efforts to explore a completely new class of

molecules as potential targets for antibacterial treatment: **"Regulatory non-coding RNA (ncRNA) networks involved in the establishment of bacterial pathogenicity**".

In the past few years, many European research groups have started to work on non-coding regulatory RNAs (ncRNAs) in a very large number of different microorganisms, mostly human pathogens. It has recently been realized that regulatory ncRNAs control bacterial pathogenicity and the adaptation of bacteria to environmental changes and to their hosts. However, these studies have only been performed in a few model organisms and are still in an initial state. It is now urgent to widen the scope of the present research to include all major human pathogens focussing on approaches allowing therapeutic interventions.

Why is the COST Action the right action?

The COST Action is undoubted the best support for this **new emerging topic** due to the diversity of approaches necessary to achieve its goal. Many groups working on different pathogenic bacteria have discovered regulatory RNAs. They now need **novel collaborations to enter this new and fast developing field**. Currently, the coordination of all essential Working Groups is insufficient. There is also a **strong need to disseminate and coordinate high throughput techniques**, which deliver a vast amount of information. The interpretation of these data requires a coordinated action to be compatible. Most importantly, many of these new European research groups working on ncRNAs are headed by **young investigators in early stages of their careers.** They need to establish strong and wide collaborative networks in order to strengthen their position in the community and thus to progress their research. Additionally, this new research area needs further smart multidisciplinary-oriented researchers. The purpose of this COST Action is to bring together these research groups in an organised and concerted way to exchange knowledge and expertise in the fields of microbiology, biochemistry, structural biology, medicine and bioinformatics. To understand how bacteria establish pathogenicity in RNA-mediated pathways, we need to study these phenomena using different and broad approaches. This goal requires **an European-wide organised structure and concerted, continuative activities**. Therefore, a concerted Action like COST is highly relevant to ensure success of this young area of research and will help young researchers to establish successful collaborations and research.

Other actions such as EUREKA, Framework Programme 7 (FP7) and similar instruments, which fund common research projects, do not adequately focus on the coordination only. The scientific projects and experiments are taking place in different laboratories. They are very favorable with the acquisition of funding for research activities both at national and international level. The important role, which this COST Action and its concerted continuative activities will play, is the conceptual approach. It will essentially promote the transfer and exchange of knowledge, experience and techniques at a variety of events, as well as the mobility of young researchers. This approach will lead to a veritable boost of new knowledge about the world of bacterial pathogenicity with the intention to find ways to face infectious diseases with novel approaches.

This COST Action is also the best instrument to prepare the basis for a network of excellence. The really exciting perspective is to establish an outstanding community, whose collaboration creates a strong synergy between all different disciplines needed to progress in the field.

B.2 Current state of knowledge

Summary of previous research

In the past decade, non-coding RNAs (ncRNAs) were discovered as a novel class of regulatory molecules. They fine-tune many processes in cells, which are required for a controlled adaptation to changing environmental conditions. These RNAs can be imbedded in mRNAs working *in cis*, they can be small RNAs working *in trans* by targeting proteins or RNAs. They can be long antisense RNAs modulating their cognate sense RNAs or like the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) RNAs (clustered regularly interspaced short palindromic repeats) inhibit the uptake of foreign DNA. Regulatory RNA elements are potent sensors of environmental signals like temperature, small metabolites, pH or salts. RNA molecules can control biosynthesis pathways by sensing the concentration of metabolites, and regulate metabolic pathways (iron metabolism, carbon utilization), which could be essential for survival of the pathogen within the host. Some of these RNAs might be required for RNA quality control mechanisms. They can regulate the expression of genes by controlling transcription, translation and/or degradation, and lately evidence is accumulating that RNA molecules can also establish resistance against phage infections (for a recent review, see Waters & Storz, 2009). In bacteria, small ncRNAs are involved

in the regulation of a growing number of adaptive processes such as quorum sensing, transition to stationary phase, iron homeostasis, the SOS response, membrane remodelling and bacterial virulence. Antisense RNAs, a class of ncRNAs that act by base-pairing to RNA targets, are responsible for the maintenance of many bacterial plasmids carrying antibiotic resistance genes.

In former European networks, a substantial effort was undertaken to discover novel ncRNAs in a few human pathogens and to search for the ncRNA targets, which are involved in establishing bacterial virulence. This was the main goal of a very successful Specific Targeted Research Project (STREP) in FP6 (acronym: BACRNAs; see http://www.projects.mfpl.ac.at/bacrnas/php/index.php). Two additional European Commission (EC) funded projects aimed at discovering regulatory RNAs in all kingdoms (RIBOREG, www.isv.cnrs-gif.fr/mc/riboreg and FOSRAK, ww.fosrak.org). Hence, ncRNAs are now an established new class that regulate most processes in all cells and in all three kingdoms. However, the characterization of these regulatory RNAs needs more fundamental work, both in understanding their expression, their synthesis, their regulation, the complexes they are involved, their structure, their targets, and most importantly the mechanism involved in their function. Understanding "**the regulation of the regulators**" will lead to a perfect picture of the circuits that control growth of living cells.

A real milestone from the BACRNA consortium was the publication of the first full transcriptome analysis of a human pathogen, *Listeria monocytogenes*, in its saprophytic and pathogenic states (see Toledo-Arana et al. Nature 459 p959, 2009). Using tiling arrays and RNAs from wild-type and mutant bacteria grown *in vitro*, *ex vivo* and *in vivo* the whole transcriptome of the pathogen was analyzed. This work provides the complete operon map of a bacterial cell and revealed far more diverse types of RNAs than anticipated. Many antisense transcripts to protein coding genes and to their 5' and 3' untranslated regions were discovered. Novel functions for riboswitches were observed and most importantly the transcriptional reshaping of the pathogen when entering the intestinal lumen was described. The transcriptional behavior of virulence genes and of pathogen-specific small RNAs was described. This work clearly defined the relevance of regulatory RNAs for the establishment of virulence in bacteria.

Furthermore, all these different types of regulatory RNAs are being found in all bacteria. The diversity of their functions is astonishing. Our network which is represented in this COST Action involves research groups working with all aspects of these RNAs: small regulatory RNAs that targets mRNAs to activate and/or inhibit transcription and/or translation, riboswitches, large antisense RNAs and CRISPR RNAs. The past few years were devoted to the discovery of novel molecules, the next steps now is to study their structure and to unravel their function.

What are the innovative next steps taken by the participants of this COST Action?

After the discovery of many novel functional RNAs, the crucial step now is to identify the targets and the function of these RNAs. The network will focus on RNAs, which are involved in the establishment of virulence and try to identify the regulatory networks, in which these RNAs are involved. The innovative steps are : i) to identify the targets of these RNAs ii) to widen the studies to a much larger group of bacteria iii) to organise and coordinate the databases with all these RNAs iii) to include studies in Archaea and yeast and iv) to study the structure and the mode of action of these RNAs in a comparative way.

In the current network, the most important and interesting human pathogens are being included. The network will be open for all European groups interested to join for studying yet additional organisms. This COST Action will aim at comparing and coordinating the studies in all these different bacteria. In addition, we have included research groups working with Archaea and yeast since it is of eminent importance to compare similar processes in other organisms to better understand the function of molecular mechanisms. We will enforce the discovery of regulatory RNAs and of their targets in all these pathogens and focus on those pathways, which play key roles in establishing virulence and developing pathogenicity. The final **academic goal is to understand the regulation of bacterial virulence** and the final **applied goal is to identify novel targets for antibacterial treatments**.

While studying the functions of ncRNAs, new RNA regulatory networks that control bacterial pathogenesis and related mechanisms like bacterial stress and environmental adaptation are being elucidated. The components of these regulatory networks will be validated to test whether they are suitable targets for therapeutic intervention. We need to analyze whole bacterial transcriptomes to

be able to understand the function of the many low abundance RNAs, whose expression patterns change dramatically upon stress. The example established by the *Listeria* studies will extremely push and advance the studies in other organisms.

Today it is no longer sufficient to study a complex process like bacterial infection using a single approach. People who are trained in many different disciplines are necessary to go from the initial discovery of pathogens, the description of the diseases to the understanding of the molecular phenomena behind infection. Therefore, the new team formed in this COST Action brings together research groups working in several disciplines (molecular microbiology, infection biology, biochemistry, structural biology, medicine and computational biology). Most prominently this consortium involves partners working in a wide variety of bacterial pathogens like: *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Vibrio cholerae*, *Helicobacter pylori*, *Legionella pneumophila* and *Listeria monocytogenes*. To enforce and enable the comparison with the role of regulatory RNAs in other kingdoms of life, we included partners working with Archaea and yeast.

This COST Action plans to address an old problem (mechanism of bacterial infection) in a novel and innovative way: it proposes to search for alternative targets for drug therapy by studying the basic principles of how bacteria adapt to changing environmental conditions through regulatory RNAs.

These groups have formerly been involved in a wide range of former European networks studying several aspects of ncRNAs. In the FP6, the EU funded several very successful projects dealing with the discovery of novel ncRNAs, their targets and their function. EU projects BACRNAs,FOSRAK, SIROCCO and RIBOREG, to mention the most prominent, have established a strong focus in RNA research in Europe. To strengthen the European action on this topic and to guarantee its sustainability. We create this new network in order to allow laboratories from 13 European countries to share information, to benefit from the expertise of the other partners and therefore establish a basic research platform indispensable for scientific progress in a novel and rapidly evolving research area.

B.3 Reasons for the Action

Reasons for establishing this COST Action and its benefits:

Because this is a new and fast developing field, which requires multidisciplinary approaches, well coordinated procedures need to be established to foster synergy, to make results comparable and to pool the data into well curated databases. Furthermore, many young researchers have entered the field, who need to establish collaborative networks in these different disciplines to reach their goals, progress their research and thus sustainable strengthen the European scientific community. The technological advances need an efficient dissemination avoiding duplication of experiments. Knowledge about infectious diseases requires a fast and professional distribution.

Major Activities and expected results:

Generally the COST scheme proposes to focus on collaborative aspects between the partners. The major activities of this COST Action will be the exchange of knowledge between the participating labs, scientific and a few soft skill workshops in large and smaller groups and further the mobility of PhD students and postdocs between the different groups. Working protocols, macromolecules, plasmids, strains and most importantly computationally cured data and the tools for the computational analysis will be made available for the partners joining the Action.

The following results are expected: **1.** Establishment of a very strong European consortium working on regulatory RNAs in bacteria, probably the dominant group world wide. **2.** Knowledge about regulatory RNAs and their targets in a large number of human pathogens. **3.** Understanding the adaptation processes in bacterial cells that lead to virulence and disease. **4**. List of potential target molecules for many different human pathogens. **5.** Determination and definition of RNA families and deposition of these in a specialized database (Rfam). **6.** Vivid mobility of young researchers and their successful placement at positions allowing a promising career. **7.** Joint publications of results.

B.4 Complementarity with other research programmes

The participants of this COST Action are already members of small national and/or international networks, however the dimension of this network is only possible in a COST Action. The major goals and objectives of the Action are unique although the research being performed is well established. This Action creates a unique frame for collaborations.

This COST action was started by a consortium working on bacterial non-coding RNAs (BACRNAs see: http://www.projects.mfpl.ac.at/bacrnas/php/index.php) funded in FP6. The success and efficiency of this network urges to expand their participants and needs novel instruments to organise this research topic in Europe. Between our application in 2004 for the BACRNA project und today the number of research groups, especially young researchers, has grown dramatically to more than 50 groups.

Researches participating in this COST Action are also working in other national and international networks. These ongoing research programmes are listed in part II-B section B.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The primary objective of this COST Action is the pooling of knowledge and coordination at the European level of research in the field of infectious diseases. The understanding of RNA-mediated regulatory networks in pathogenic bacteria requires concerted studies in different organisms including Archaea and yeast. Data from different organisms should be made available and comparable through the usage of compatible techniques and approaches. Finally, strategies used by pathogens to adapt to their hosts are to be deciphered with the aim to identify novel targets for therapeutic intervention.

C.2 Secondary objectives

1. Compatibility of experiments and results in the field of regulatory RNAs in various human pathogens.
2. Comparison of results on a European scale.
3. Establishing a strong European wide BACRNA community identity in a multidisciplinary field.
4. Creation of a bacterial RNA database.
5. Dissemination of knowledge via Wikipedia.
6. Vivid mobility of young researchers and their successful placement at positions allowing a promising career.
7. Joint publications of results.

C.3 How will the objectives be achieved?

To study the role of regulatory RNA in bacterial pathogenicity a multidisciplinary approach is essential. In addition, the development of methods required for these studies is very fast and the accumulation of vast amounts of data needs special care and special attention. Clinical microbiology describing the diseases and isolating strains, methods of growing pathogens, sequencing, transcriptome analysis, proteomic analysis, genetics and biochemical /biophysical approaches are nowadays "a must" to explore scientific questions of these dimensions. This COST Action will bring together people with very diverse expertise in addition to making all different human pathogens available for comparing purposes. Experiments should not be repeated in different organisms if not necessary. Nowadays a lot of unpublished data accumulate in different laboratories. Through such an Action, all groups will be informed what is being done in the different laboratories or what has already been done. The network will be very complementary and the groups will not compete with each other because different organisms and different approaches are represented in the network.

1. **Transfer of knowledge:** Many research groups working with different pathogens recently discovered that some of their key factors they were searching for are RNA molecules. Working with RNA requires different approaches and know-how and these groups urgently need to learn from expert RNA groups how to work with RNA.

2. **Support of young researchers**: This novel and exciting area of research attracted many young researchers in the early stages of their careers. This network represents an ideal instrument for these young groups to find their collaborative partners in many different disciplines required to successfully address their scientific problem and to offer or mediate a promising and continuative next position.

3. **Impact:** This COST Action will help European academics and its society in several aspects: the coordination of the research will allow optimal usage of infrastructure avoiding duplication of experiments and infrastructure facilities. Very expensive research facilities in the following techniques will be available for the network partners: deep sequencing, microarrays, proteomics, mass spectrometry and bioinformatics.

4. **Creation of a database:** results will be deposited into the Rfam database; RNA sequence database roughly equivalent to UniProt in the protein world and an RNA alignment archive will be coordinated.

Scientific & Technological advances:

The Action will clearly have an impact at advancing science and technologies in Europe. This Action stands at the beginning of a new and very fast expanding field of research. It urgently needs coordinated activities to avoid duplication and incompatible technologies. The added value lies in making research in different organisms compatible, comparable and exchangeable.

The transcriptome analysis of many bacteria will be made comparable, the regulatory networks leading to the establishment of virulence will be known in many different pathogens; the comparative approach will speed up the process in many new organisms; the diversity of strategies

used by nature in different organisms will become evident; technological advances will be disseminated in a controlled way avoiding errors and duplications. Most importantly, the compatibility of the different approaches will boost the usage of date for complex analyses necessary for the field of systems biology.

Scientific exchange: The objectives of this COST Action will be achieved by enabling scientific exchange in a coordinated and organised way. The different working tasks, which will be performed for all pathogens being studied, will be set up systematically proposing an "assembly line" of approaches and techniques. The four Working Group Leaders will organise and overview the methods, experimental details and tools, which are used to analyse the regulatory RNAs and targets from different pathogens. All these technical information will be made available to all the partners through a restricted area on the website dedicated to this COST Action. This will make the data comparable and much better interpretable.

Mobility of young researchers: A major focus will be put on the mobility of young researchers, research exchange in between the groups and placement at promising positions. A large part of our budget will be allocated to the travelling of young researchers between the different laboratories to learn the techniques hands on by experienced groups, where the techniques are established. This is a win-win situation for the young researchers, the working teams and the Working Groups as a whole. This is a much more successful and efficient way than organizing courses with many participants. On the website dedicated to this Action we will provide links to practical workshops that are organised in Europe and where some of the partners are involved in the organization (such as the "Computational Methods for RNA Analysis" Benasque, Spain).

Creation of a tool box: A tool box with all the techniques and protocols will be exchanged and made available via the restricted area of the Action's website, including detailed advices. Some protocols are already published and will be available on the non-restricted website area dedicated to the COST Action.

Creation of a strain and plasmid collection: Pooling the strains and plasmids existing in the network will greatly facilitate exchange and availability of these tools and contribute to the compatibility of experiments. It will be collected via the restricted area of the Action's website.

Creation of a database: In addition to depositing already well analyzed and classified RNAs into the Rfam database, we will create an RNA sequence database roughly equivalent to "UniProt" in the protein world and an RNA alignment archive.

Events and workshops: Common meetings will be organised annually for all participants. In addition, after the general meeting, Working Groups will organise workshops for the young researchers (PhDs and postdocs) for exchange of experience with different methods (i.e. methods for gene inactivation in various bacteria species; high throughput methodologies such as deep sequencing, tiling arrays; bioinformatic tools to analyze RNA structure folding and RNA structure alignments, to predict ncRNA-mRNA interactions). Additionally some soft skill workshops will be offered such as "How to provoke enthusiasm about scientific results", "How to find the dream job" and "How to successfully acquire funding for great scientific project ideas".

C.4 Benefits of the Action

1. Dissemination of knowledge about the role of ncRNAs from a few model organisms to a long list of human pathogens. This knowledge will help national institutes to increase their research potential in this domain.
2. A strong and active COST Action on this topic will encourage funding organisations concerning this topic. Further progress will have essential impact on the health of Europe’s citizens.
3. European early-career researchers will be encouraged to participate and to increase their mobility and career perspectives.
4. The methods used in the different labs will thus become available to the Action's participants.
5. Merging research on bacteria with Archeae and yeast will provide novel aspects and foster unexpected discoveries.

6. This COST Action will help young group leaders in building up their own independent careers by obtaining mentoring relations with experienced senior scientists and by establishing strong collaborative networks.

C.5 Target groups/end users

The usage of the outcome of this COST Action will be manifold:

1. Academia will profit from the strategic organization of the research field for teaching, for writing textbooks, etc..
2. Whenever additional pathogens will be studied, or discovered, a working plan for how to address and find the important molecules and pathways in an organism will be available in a tested, evaluated form.
3. Pharmaceutical companies might want to try targets identified by this project for therapeutic intervention.
4. General public: since the ultimate goal of this Action is to prevent bacterial infections, the general public will definitely profit from this Action.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

The scientific aims of this COST Action are ambitious and require different approaches from different disciplines. The unifying aspect of the group is its common goal: to understand how bacteria adapt to changing environmental conditions by the action of non-coding RNAs. The main focus will be on the adaptation process of the bacterial cell to the host during virulence. At the very focus of the Action are the regulatory RNA molecules that control these adaptation processes.

Research tasks to be coordinated by the COST Action:

1. **Identification of non-coding RNAs (ncRNAs)**. NcRNA have been established in the last years as important macromolecules that fine-tune adaptation processes in bacterial cells. The discovery of ncRNAs was originally via genetics, but nowadays there are several approaches which can be used. These are: **a.** computational predictions followed by detection of the predicted RNAs via Northern blotting or Real-Time PCR (RT-PCR); **b.** Co-immunoprecipitation with antibodies to proteins that bind to regulatory RNAs, like Hfq; **c.** deep sequencing of cDNAs isolated under different conditions of infection; **d.** hybridization of tiling arrays with RNAs isolated under conditions of infection and **e.** genomic Systematic Evolution of Ligands by EXponential Enrichment (SELEX) for the detection of genomic RNA aptamers with high affinity to ligands of choice.

   All these approaches have been used by partners in the network and will be available. Advices will be provided to groups inside the Action but also to any other new groups, which intend to isolate ncRNAs in novel bacteria or other organisms. Our Action is open to any other countries, which want to join it.

   The following pathogens are being worked with in this COST Action: *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Yersinia pestis, Shigella, Streptococcus pyogenes, Vibrio cholerae, Helicobacter pylori, Legionella* and *Listeria monocytogenes*.

   Because it is of eminent importance to compare bacterial strategies with those of other organisms, this COST Action will include people working with Archaea and yeast. These organisms are significantly different from bacteria but encounter similar stresses and challenges during their life cycles. These organisms have always been very useful for certain approaches, like for the isolation of stable proteins or for genetic screens, which might be worth using. Regulatory RNAs are less well studied in these organisms and it is expected that they will play different roles than in bacteria.

2. **Characterization of regulatory pathways and networks in which these regulatory RNAs are involved.** Regulatory RNAs can target other RNAs, most of them targets the 5' UTR of mRNAs, but they can also target coding regions, 3' UTRs, small RNAs or potentially even antisense RNAs. In addition, ncRNAs can target proteins and regulate their activity, like the 6S RNA, which interacts with RNA polymerase regulating transcription. The major effort after discovering novel RNAs is to identify their targets and the pathways they are involved in. Again, computational predictions have initially been difficult, but the programs are improving rapidly, so that predictions are getting better and better. In addition, biochemical methods can be used for isolating larger particles containing the ncRNA, its target and other important proteins necessary for the functioning of the regulation. All these techniques are available in a few partner laboratories and will become accessible to the whole group.

3. **Studying the mode of action of regulatory RNAs, their synthesis and decay.** Most regulatory RNAs are expressed under specific conditions and their abundance can vary substantially. Methods to determine the amount of low abundant RNAs are tricky and special expertise is required to handle this problem adequately. Expression conditions and the regulation of the expression of the regulatory RNAs have not yet been addressed extensively. This is an important aspect because when the signals that activate these RNAs will be identified, it might be possible to find antagonists of these signals as a means to avoid infection. Little is know about how these RNAs are degraded and why and when small RNAs are more or less stable. The RNases involved in the processing and decay of small RNAs or other regulatory RNAs need further analysis.

4. **Characterization of ncRNA-protein complexes and ncRNA machines.** Little has been done until today to find out whether these RNAs work in conjunction with proteins and which additional factors are required. Gram positive and Gram negative bacteria might differ substantially in this aspect. The global regulator protein Hfq has been found in a few bacteria, like *E. coli* or S. *typhimurium* to be essential for RNA-RNA regulations. However, in *Staphyloccus aureus*, Hfq does not seem to be necessary for the activity of small RNAs, although it contains a gene for Hfq, but in a shorter version. Other bacteria, like

Spreptococcus pyogenes, do not contain a gene coding for Hfq. Efforts will be undertaken to identify the proteins interacting with small RNAs. The localization of regulatory RNAs in bacterial cells is a difficult task, but it will be essential to determine at which level of gene expression they interfere. They might assemble with the transcriptional machinery or with the ribosome or any not yet identified macro-particle (RNP complex). In their environments including the host, bacteria are forming heterogeneous populations of cells that communicate with each other and respond to specific external signals. How often within a population these RNA-dependent regulatory events occur in a stochastic way remains to be addressed. We will also move from regulation in a cell population to single cell in time.

5. **Structure determination of the ncRNAs**. To understand the mode of action of functional RNAs, it is essential to know their structure. Knowledge about structural motifs that govern RNA folding is growing rapidly allowing more and more accurate predictions. It might not be necessary to have a x-ray crystal structure from each RNA, but knowing its secondary structure and which domains are accessible for base pairing with target mRNAs is important.

   In a first approach, a newly discovered RNA will be compared with homologous RNAs in other organisms. A phylogenetic comparison will give a first suggestion of its secondary structure and of the conserved (and important) domains of the RNA. Then chemical probing *in vitro* will help to model a precise secondary structure. This method will gain knowledge on the binding sites of the ncRNA ligands and monitor the conformational changes, which might occur in the ncRNA upon binding. *In vivo* structural probing will analyze the folding state of the RNA under different conditions and will complement the *in vitro* probing. X-ray structure determination, if possible in complex with its target molecule and other specific proteins is the ultimate goal, which has not yet been achieved for any small RNA. Only for riboswitches, the x-ray structures of the aptamer domains for several of them in complex with their small ligands have been solved. This might be due because small RNAs have been discovered quite recently and also because they might not have a rigid well folded conformation, but might have to be flexible to facilitate interaction with different targets. In this network all these techniques are available and know-how about RNA structure is best represented in this group.

6. **Determination of RNA families**. Many regulatory RNAs are conserved among different organisms and it is expected that these RNAs will have a similar function. Because functional RNAs are more often conserved by structure than by sequence. This often makes it difficult to compare or align RNA molecules. Therefore efforts are being undertaken to develop tools not only to align RNA sequences but also to align them by structure. Once a group of similar RNAs have been found to have similar structures and functions they are assigned to a new RNA family. Only recently, a new depository database "Rfam" has been established, similar to the Pfam database for proteins, were RNA families are described. This is a very useful tool to compare newly identified RNAs to be able to assign them a structure and a function. This COST Action will be strongly linked to the Rfam database by the participation of Paul Gardner, who heads this database at the Welcome Trust Sanger Institute.

7. **Validation of targets for therapeutic interventions: model systems for testing drugs**. Finally, to boost the pharmaceutical interest in our project, this Action will develop animal tests to validate several targets identified in the coming years for therapeutic intervention. Genetic knockouts of small RNAs or the regulatory elements will in a first step give information whether they are essential for the establishment of virulence.

Structure of the work plan

The many different approaches necessary for this Action will be allocated to 4 Working Groups specialized in the different disciplines (microbiology, molecular biology, biochemistry and computational biology). These Working Groups will be responsible for coordinating the knowledge, methods and material available in the network and necessary for best planing experiments. All participants have access to all four Working Groups depending on which aspect of the research they will work on. Any new participant should have, once they conform to the NoU, the possibility to join the activities of the Working Groups.

D.2 Scientific work plan methods and means

Workplan

The above mentioned research tasks will be organised into four Working Groups. Each Working Group will be headed and coordinated by an expert belonging to the Management Committee. The network has a very wide range of expertise and the methods and infrastructure available are listed shortly below. The main experts are mentioned to give a better picture of the strength of the network. For a better picture of the qualification of the participants, please see part II-B, Section A.

Working Group **I (headed by N.N.): Identification of ncRNAs and their targets** will coordinate the comparison and set up the methods for the isolation of novel ncRNAs and their targets and coordinate together with Working Group IV (computational biology) the data curation and storage for the whole network. This will be particularly helpful to identify common and species-specific RNA regulatory dependent networks.

Working Group **II (headed by N.N.): Mechanisms of regulatory RNAs and RNA structure** will emphasize on the mode of actions of the ncRNAs and on the regulatory mechanisms connecting ncRNAs to their targets, on the ncRNA/protein complexes required for the function of the ncRNAs, and will analyze the structure of defined regulatory complexes.

Working Group **III (headed by N.N.): Bioinformatics and computer sciences.** The world most prominent researchers in the field of structural prediction and RNA structure modeling will provide the network with all tools required to keep, store and manage the data and most importantly will provide state of the art computer programmes for the analysis of large sets of data both from sequencing (identification of ncRNAs) but also for structural and functional analysis.

Most importantly, the classification of RNAs into families (Rfam) will greatly facilitate the assignement of newly discovered RNAs. The coordination and joint comparison of RNAs from different organisms will also greatly facilitate the identification of new families. This part of the work will be coordinated by Paul Gardner. The depository of a novel RNA family in the Rfam database can be coordinated with a publication in the Rfam section in the RNA Biology journal.

Working Group **IV (headed by N.N.): Microbiology and host-pathogen interactions.** This Working Group will coordinate the microbiological know how necessary for cultivating strains, obtaining clinical isolates and strains, cultivating techniques. In addition, model systems for the analysis and validation of targets will be coordinated and compared. There are several groups working in hospitals and having access to patients and to collections of clinical isolates.

Human resources and technical infrastructure

The uncontestable advantage of this COST Action is the wealth of knowledge, human potential and infrastructure, which will be pooled in this consortium. This will make the strongest and most efficient group of researchers working on bacterial regulatory RNAs world wide.

E. ORGANISATION

E.1 Coordination and organisation

Structure, Competencies and Responsibilities. The Management Committee (MC) is composed of the Chair (C) and the four Working Group Leaders (WGL) and the Administrative Manager (AM). The main responsibilities are as follows: the C monitors the progress of the main course of the Action and discusses possible corrective activities with the WGLs during the year. The WGLs monitor the progress of their individual Working Groups (WGs), the quality and impact of the collaboration with the other three WGs and discuss possible corrective activities with the other WGLs and the C. The AM organises, coordinates and co-executes all networking and management activities in accordance with the annually approved detailed COST Action activities, the basic PR-concept, the COST requirements, in close collaboration with the C. The AM also executes soft skill workshops for young researchers. The individuals of this group of six agreed to their roles and responsibilities. The individual Research Teams are responsible to contribute according to the planned and approved Action. The detailed definition of the responsibilities and competencies is described in the document COST 299/06 "Rules and Procedure for Implementation COST Actions".

Organisation and Coordination. The official election of the MC will be organised at the kick-off meeting of the Action. All participating research teams will attend this meeting where the following will be confirmed and decided, respectively: a) the main course of the Action, will be presented to the participating Research Teams and confirmed; b) the coordination of the Research Teams of each working group as well as the working groups among each other concerning the exchange of existing and new data, the exchange of young researchers will be presented, discussed and decided; c) the mentoring programme will be started; d) the content and function of the Action's website and its restricted area as well as its maintenance will be presented, discussed and decided and e) the joint events and workshops, which will be organised and implemented in the next year will be presented, discussed and decided.

The MC will meet annually. These meetings will be organised in order a) to present achievements; b) to reassess the Action's activities and its impact; c) to decide on additional experts due to previous calls; d) to decide the modifications and joint events and workshops and e) to decide on research funding applications and research project ideas, which are suitable for all Working Groups or a selection of Research Teams of the Action and which will be accordingly submitted to national, European and/or international funding commissions. With her long-term experience the AM will support these teams during the project creation and writing process. The MC members will be in regular phone and email contact in order to monitor the course of the Action. The WGL will be in regular phone and email contact with their Research Teams and the other WGLs in order to individually decide scientific exchange between the Teams and Working Groups as well as the mobility of young researchers.

A call for a consultant for public relations (PR) will be published. The sub-contracted PR-consultant will develop a detailed PR-concept of networking activities for the full duration of the Action. This PR-concept will be presented, discussed and decided at the kick-off meeting. On the basis of the approved PR-concept, the AM will develop a roadmap and accordingly organise, coordinate and co-execute its activities.

The following milestones are set to monitor the main course of the project:

- M1: Kick-off Meeting.
- M2: Approved main research and networking course of the Action.
- M3: Approved basic PR-concept.
- M4: Approved training-concept for young researchers.
- M5: Website ready to further develop for the need of the participants of the Action.
- M6: Each mentee is supported by a suitable mentor.
- M7: Annual Meeting in Year 2 according to the agenda a) to e) listed above.
- M8: Annual Meeting in Year 3 according to the agenda a) to e) listed above.
- M9: Final Meeting in Year 4 according to the agenda a) to e) listed above.

Individual collaborations between Research Teams are already established at national and/or international level (see Section B.4). This is also expressed in a number of joint publications. The annually organised international conference "RNA Biology" and workshops such as "RNA Bioinformatics" and "RNA Ontology" are an important basis to further networking activities between the involved Research Teams. Workshops dedicated to techniques and methodologies such as sequencing, microarrays, proteomics, mass spectrometry and bioinformatics were launched for young investigators so that techniques and methodology exchange has been cultivated. Its further fostering will "synergize" the collaboration between the Research Teams.

E.2 Working Groups

As already stated earlier the WGLs will be in regular phone and email contact with their research teams and the other WGLs in order to individually decide scientific exchange between the Teams and Working Groups as well as the mobility of young researchers. Additionally they will regularly meet at international conferences and workshops. These events will be announced on the Action specific website.

WG 1 Identification of ncRNAs and their targets: This group will coordinate and overview both the technologies used to discover ncRNAs and their targets as well as the organisation of the data storage in conjunction with WG III.

WG 2 Regulatory pathways, mode of action, and structures: This group will mainly discuss and compare the results obtained in the different organisms concerning the mechanisms of action, the molecular networks. Common discussion and data comparison will be the main action in this WG.

WG 3 Bioinformatics: This group will have the important task to develop and make available the algorithms for data analysis, data storage and classification of molecules after structure and sequence comparison. The bioinformatics group is very strong and is connected to or leading the main bio-computational activities in Europe concerning RNAs.

WG 4 Microbiology and Disease: This group will coordinate strains and plasmids, cultivation techniques, disease related questions and the target validation experiments.

E.3 Liaison and interaction with other research programmes

The Working Group members of this COST Action competitively acquires - as coordinator or participant - remarkable funding at national, European and international level. The ongoing research programmes, listed in part II-B, section B, are a highly qualified basis of this COST Action to accelerate the consolidation of the multidisciplinary and intersectoral international knowledge in the field of regulatory RNAs in bacterial pathogenicity.

With the start of this COST Action the MC will get in touch with all coordinators of the ongoing research programmes (see part II-B) and discuss suitable interactions in order to jointly create a sustainable concept information exchange. Additionally the AM will regularly collect planned events such as topic specific conferences, workshops, seminars, etc. at all coordinators' places and publish on the website. Thus the individual programmes and actions will reach a broader research

audience. The members of the COST Action will be motivated to actively participate in the "European Researchers Night" action. Hence, an extremely broad public could learn playfully more about the latest research results as well as about suitable measures of handling with bacterial pathogens and its relevance in the 21st century in Europe and world-wide.

E.4 Gender balance and involvement of early-stage researchers

This network has an excellent gender and age balance. The MC is composed of four women and two men. One half is in young (definition see paragraph below) and the other half in principal researcher positions at their organisations. This composition represents and substitutes the early-stage researchers' interests and ideas alike it influences the decision making with its members' long-term scientific and management experience.

Approximately one third of the participants belongs to the group of young researchers who are in the first 5-10 years of their independent research. Many of them just came back from postdoc positions abroad and are in the process of establishing their own research groups. This COST Action will ease their career development with important contacts to experienced and successful researchers in the fields.

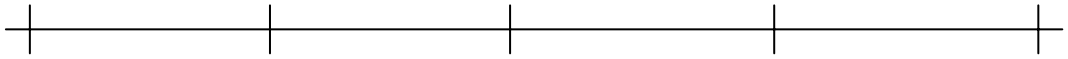
The mentoring programme, which will start with the kick-off meeting, will additionally be organised to address specific issues such as:

- Developing relationships, creating trust and building confidence
- Setting clear objectives
- Handling emotions and dealing with conflict and other difficulties

This mentor-mentee pairing will be supplemented with soft skill workshops, which are offered at the individual institutions as well as with this COST Action.

These actions will help young researchers to build up their collaborative networks as well as an efficiently acting work group, both highly essential for a successful research.

F. TIMETABLE



Year 1 (Preparatory work month 1-2):

- Develop detailed PR-concept.
- Develop training-concept for young researchers.
- Develop mentoring programme.

Year 1 (Kick-off meeting months 3):

- Decide main research and networking course of the Action according the Research Teams' presentations.
- Decide detailed PR-concept.
- Decide training-concept for young researchers.
- Decide mentoring programme.

Year 1:

- Implement decided research and networking course of the Action.
- Develop the PR-roadmap on the basis of the decided PR-concept and implement it.
- Implement the training-programme for young researchers.
- Implement the website ready to further need of the participants of the Action and regularly update it.
- Mentor-mentee pairing and decide on individual mentoring programme.
- Entry of identified RNA families to the Rfam database.
- Publish the call for new experts (with focus on Clinicians) and invite a close-pitch selection for research presentation at the annual COST Action meeting.

Year 2:

- Annual COST Action meeting and decide on the accommodation of new experts in the COST Action.
- Implement reassessed research and networking course of the Action.
- Implement the reassessed PR-roadmap.

- Implement the training-programme for young researchers.
- Update the website according to the needs of COST Action members.
- Continue mentor-mentee pairing and decide on individual mentoring programme.
- Entry of identified RNA families to the Rfam database.
- Publish the call for new experts (with focus on Clinicians and pharmaceutical SMEs) and invite a close-pitch selection for research presentation at the annual COST Action meeting.

Year 3:

- Annual COST Action meeting and decide on the accommodation of new experts in the COST Action.
- Implement reassessed research and networking course of the Action.
- Implement the reassessed PR-roadmap.
- Implement the training-concept for young researchers.
- Update the website according to the needs of COST Action members.
- Continue mentor-mentee pairing and decide on individual mentoring programme.
- Entry of identified RNA families to the Rfam database.

Year 4:

- Annual COST Action meeting.
- Implement reassessed research and networking course of the Action.
- Implement the reassessed PR-roadmap.
- Implement the training-concept for young researchers.
- Update the website according to the needs of COST Action members.
- Continue mentor-mentee pairing and decide on individual mentoring programme.
- Entry of identified RNA families to the Rfam database.

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, DK, FR, DE, EL, IL, IT, NL, PT, ES, SE, CH, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 52 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

Since the major objective of the Action is both urgent and complex a broad audience needs to be informed as well as involved.

1. Additional basic researchers might bring new aspects into the Working Groups.
2. Cooperation with existing and new established research frameworks will "synergize" the Action.
3. Inclusion of clinicians, biotech and pharmaceutical industry will create the platform for translational research.
4. Information towards the public bodies will create a communication platform between science and politics.
5. Information towards the general public will contribute to knowledge.

H.2 What?

The following variety of different tools will reach the basic researcher in the field alike broad public:

1. A *website* will be established with the aim to become "the communication platform" for bacterial pathogens and RNA biology. On this platform international scientific events, activities, workshops, publications, animations about basic mechanism and knowledge about bacterial pathogens (topics see under "folder"), a prominent link to the *database for non-coding RNA families (Rfam)* and links to other research frameworks will be published.
2. A *restricted area on the website* will be created for the access of involved Researcher Teams to the tool box and database.
3. *Scientific publications* (reviews) and publication of workshop reports will be created and published on the website.
4. A *special focus* on the adaptation of bacterial pathogens to constantly new environment will be serially published in the peer-reviewed scientific journal *"RNA Biology"*.
5. A *folder* will be handed to policy makers, stakeholders and at prominent places such as hospitals, doctors, health insurance funds, universities and others. It will include links to the website of the Action, to other research programmes on the RNA topic and particularly information about basic mechanisms such as what is splicing, what is a bacteria, what is stress for a bacteria and what is its reaction on such stress, how makes this organism sick. This folder will be translated at least to the main if not all languages of the participating countries.
6. A special focus on the adaptation of bacterial pathogens to constantly new environment will be published in *journals for a broader public audience*, which will be disseminated in several European languages such as the journal "research.eu".
7. *Science website platforms* suitable for either a scientific or/and a broader public audience such as http://cordis.europa.eu/wire, http://www.scinexx.de, http://seedmagazine.com, http://www.reuters.com and http://esciencenews.com will be updated regularly with new results, generated during the course of this Action.
8. *TV and radio programmes* with scientific focus will be contacted and offered material generated during the course of the Action.
9. "Hot results" will be published at least in the *daily press.*

10. *Regular personal contacts* to the coordinators of the different research frameworks will be established with the start of the Action in order to regularly exchange information and knowledge.
11. *Networking* on all Action related issues at *European and international scientific events and conferences* in order to bring new views and disciplines into the Action to enrich the growing network.
12. In the course of this Action *additional basic researchers, clinicians* and *biotech companies will be invited to apply, present their work and to participate in the Action.* The exchange of knowledge and experience between basic and clinical research as well as the inclusion of genetic engineering might generate new insights to the complex topic of adaptation of bacterial pathogens to new environments. The *pharmaceutical industry* will be invited if suitable outcomes are available.
13. Consultants and advisors will be invited as soon as Intellectual Property Rights and ethical aspects become important.

H.3 How?

All sorts of dissemination tools (see H.2) will be used to keep the scientific as well as the public society informed and curious. A detailed coherent Public Relation concept (PR) will be developed with the start of the Action in order to ensure a concerted information action.

1. *Additional basic researchers bring new aspects into the* Working Groups*:* It is essential to further the translational network activities. The core group of this Action is composed of *basic researchers* who work in the multidisciplinary field of RNA biology. This group will be supplemented according to the generated results during the course of the Action.
2. *Cooperation with existing and new established research frameworks:* With the start of the Action existing research networks in the field will be invited to coordinate their activities with this Action in order to "synergize" all different sorts of activities.

3. *Inclusion of clinicians, biotech and industry:* In the course of this Action *clinicians* and *biotech companies* will be invited to apply and to participate in the Action. The exchange of knowledge and experience between basic and clinical research as well as the inclusion of genetic engineering might generate new insights to the complex topic of adaptation of bacterial pathogens to new environments. The *pharmaceutical industry* will be invited if suitable outcomes are available.
4. **Deposited ncRNA families in the Rfam database goes WIKIPEDIA!** The scientific results will of course be published in scientific journals but more important for this action is the storage of sequence data in already existing and new databases. The deposit of these data in the Rfam database is highly suitable for this purpose. Beginning 2009, the Journal RNA Biology started a new section called "Rfam" for RNA families together with the Wellcome Trust Sanger Institute in order to motivate scientists to deposit their RNA data in the public data base on RNA families. See http://nar.oxfordjournals.org/cgi/content/abstract/gkn766v1 . This project was started by the proposer of the COST Action, who is also the Editor in Chief of RNA Biology. See http://www.landesbioscience.com/journals/rnabiology/article/7635/ . Paul Gardner, the Editor of the Rfam section is also the head of the Rfam project. The innovative issue for a broad dissemination is that whenever a new family of RNA molecules is described and stored in the Rfam database a corresponding entry in the online encyclopedia **Wikipedia** occurs simultaneously. This project called a lot of attention in the scientific community.

   See http://www.nature.com:80/news/2008/081216/full/news.2008.1312.html. This effort will ensure a highly important archive for well curated sequence alignments and secondary structures for all researchers in the community since Wikipedia is an open access online platform. It eases the access to data tremendously.
5. *Information towards the public bodies as well as the general public:* The reason why infectious diseases are the second-ranking cause of death worldwide is also based on the fact that the correct education in many countries fails. New insights to the adaptation of bacterial pathogens could also bring new insights how to behave in order to avoid infections and re-infections. Therefore it is important to inform and transfer existing and new knowledge about bacterial pathogens to regional planners, health careers, stakeholders and policy makers as well as to a broad public audience.