European Cooperation in Science and Technology
- COST -

Secretariat

Brussels, 9 June 2011

COST 4115/11

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action BM1102**:** Ciliates as model systems to study genome evolution, mechanisms of non-Mendelian inheritance, and their roles in environmental adaptation

Delegations will find attached the Memorandum of Understanding for COST Action BM1102 as approved by the COST Committee of Senior Officials (CSO) at its 182nd meeting on 17 May 2011.

MEMORANDUM OF UNDERSTANDING

For the implementation of a European Concerted Research Action designated as

COST Action BM1102

CILIATES AS MODEL SYSTEMS TO STUDY GENOME EVOLUTION, MECHANISMS OF NON-MENDELIAN INHERITANCE, AND THEIR ROLES IN ENVIRONMENTAL ADAPTATION

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 4154/11 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 to establish a network of ciliatologists in order to strengthen and consolidate European research in this area aiming at deciphering the molecular mechanisms underlying epigenetics and non-Mendelian inheritance and environmental adaptation.

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 40 million in 2011 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 IV of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

Darwinian selection of random mutations is considered the driving force for evolution. However, it is now clear that acquired characters can also be transmitted from one generation to the next through non-Mendelian inheritance, with influence on cell differentiation and occurrence of diseases. Important questions are whether environmental changes can induce such epigenetic variation and if these variations drive adaptation. Research on ciliates has greatly contributed to unravelling the molecular mechanisms of non-Mendelian heredity. These unicellular eukaryotes constitute excellent models to study basic biological processes. We propose the organization of an innovating, highly interacting, dynamic European research network focusing on epigenetics, genome evolution and ecology of ciliates. The aim of this COST Action is to obtain solid evidence for the role of epigenetic phenomena in environmental adaptation. Participants will be from 9 COST countries, with the possible addition of non-COST countries for mutual benefits. A European network of ciliate molecular biologists will improve the quality of the research and training of young scientists on the continent, and will provide greater visibility for European research. The knowledge expected to be produced through the Action will likely be of use to other researchers in the fields of genetics, genome evolution, population genetics, environmental sciences, cell differentiation and health.

Keywords: Epigenetics, comparative genomics, environmental changes, molecular adaptation, DNA rearrangements.

B. BACKGROUND

B.1 General background

How living organisms adapt to environmental change is a fundamental question in biology with important implications for agriculture and medicine, especially in the current era of global warming. Modern biology has gone a long way to explain the evolution of genomes through the Darwinian selection of random Mendelian mutations and has largely rejected Lamarck's concept of the inheritance of acquired characters. However, the fast-developing field of epigenetics has revealed (i) that environmental changes can induce epigenetic modifications that affect gene expression and

(ii) that these modifications can be transmitted across sexual generations i.e. are not necessarily erased at meiosis. Although it is now clear that experimentally induced epigenetic changes are transmitted to sexual progeny in some species, including plants and ciliates, it remains to be determined how prevalent such processes are in nature. The objective of this COST Action is to build a research network focussing on epigenetics, genome evolution and ecology of ciliates, with the aim to provide a solid demonstration of the role of non-Mendelian inheritance in environmental adaptation.

A COST network is highly desired to pursue this research topic. In Europe, ciliate research is performed by individual groups scattered across the continent, with different and complementary expertise and often using different ciliate species as models. A comparative approach will allow the Action to forcefully address key questions related to DNA rearrangements, RNAi, chromatin modification, genome evolution, speciation and molecular adaptation in eukaryotes. A network that allows European integration of national research projects addressing diverse dimensions of the topic at hand is the best approach to solving such an important and highly interdisciplinary problem. Frequent exchange of knowledge and expertise in the field, as well as the latest technological breakthroughs, will foster the formation of scientific excellence. Most importantly, the highly interactive and flexible features of the COST Action will boost the development of European ciliate molecular research by injecting new blood from all over the world. Young researchers will be trained and equipped with new skills to ensure that they are technically and intellectually competitive in the global environment.

Last but not least, this very first COST network in ciliate epigenetic research will help launch further cooperative and research-based projects at the European level, such as those of the EU Framework Programme which will be actively discussed and attempted. A COST Action that can be extended to research groups not initially involved provides the best platform for healthy and prospective growth of the entire ciliate community in Europe.

B.2 Current state of knowledge

Ciliates are unicellular eukaryotes found throughout the planet's waters. The complex cell structure, fast division rate and easy cultivation of many species have made them very useful models, and their phylogenetic position – at an equal distance from plants and animals – offers a unique perspective on basic biological processes. It may be true that ciliates were regarded as rather obscure organisms for many years, but, as Francis Crick wrote over 30 years ago to one of the participants of this COST Action "no doubt, ciliates are not quite the same as most other eukaryotes, but then the phages are not the same as a typical prokaryote. This has not meant that they have proved of no value for fundamental biological research. Ciliates may prove to be equally useful." This has been impressively demonstrated by two Nobel prizes awarded for work on ciliated protozoa, for the discovery of catalytic RNA in 1989 and for the discovery of how chromosomes are protected by telomeres and telomerase in 2009. Ciliates have also provided outstanding models for epigenetic inheritance, with pioneering studies of prion-like structural inheritance in *Paramecium*, of histone modifications in *Tetrahymena*, and of the role of small-RNA pathways in the epigenetic programming that occurs during development, in both *Tetrahymena* and *Paramecium*. In each case, the phenomena and the mechanisms first described in ciliates have proven to be of widespread occurrence and of fundamental importance for the biology of eukaryotes.

In higher organisms, small RNAs are used to mark and "silence" the genomic regions which otherwise could bring about deleterious effects. A similar strategy has also been applied by ciliates, but with a twist: ciliates have evolved a unique way to separate germ-line and somatic functions, nuclear dimorphism. A diploid micronucleus is only involved in sexual exchange, while a polyploid macronucleus is responsible for all gene expression but is discarded during sexual processes. During the development of a new macronucleus, massive DNA rearrangements produce a "streamlined" version of the germ-line genome ready for expression. A large portion of the germ-line DNA, including all the potentially deleterious repetitive sequences that were long considered "junk", is eliminated, while all the genes required for the survival of the organism throughout its life cycle are amplified to a high ploidy level. The same germ-line genome can be rearranged in different ways, producing alternative phenotypes. Although purely somatic, these 'mutations' are inherited in sexual progeny in a non-Mendelian manner.

Recent studies have uncovered the roles of different types of non-coding RNAs in non-Mendelian inheritance in ciliates. In some species, genome-wide germ-line small RNAs produced during meiosis perform a natural genomic subtraction, enabling selected molecules to reproduce the parental DNA elimination patterns in the developing zygotic macronucleus. This occurs via the targeting of histone modifications, similar to small RNA-directed heterochromatin formation in other eukaryotes. In other species, long transcripts from the parental macronucleus act as templates to guide the unscrambling of genes during development. In all cases, the non-Mendelian inheritance of alternative somatic genomes may be viewed as a buffer allowing short-term adaptation to rapidly changing environmental conditions, while preserving the germ-line genome for the long term.

An enormous challenge that remains to be solved is to unravel the interactions between environment and the epigenetic inheritance systems. The single cell body of ciliates is naturally exposed to the changing factors of the environment. Temperature, for instance, has been a great selective power during the evolutionary history of ciliates. Thermal stress is known to alter cellular components and structures of organisms.This is also true for ciliates, which provide a unique opportunity to explore the first cold-adapted eukaryotic genome. Comparison of the transcriptome and genome of the cold-adapted species with data obtained from closely related species from milder environments has led to exciting findings on molecular adaptation to cold. A further objective is to understand if the epigenetic systems are also modified in species living under different temperatures and how these modifications favour survival.

The scientific core of this COST Action is exploration of RNAi-mediated genome rearrangement mechanisms in different ciliate species, with special emphasis on identification of non-coding RNAs and protein players, in order to establish the connection between this process and the different somatic genome patterns among ciliate lineages. Inter-species comparison at different levels will strengthen understanding of epigenetic inheritance systems in eukaryotes. The efficient exploration of these questions requires further development of the ciliate models and close collaboration among research groups in different research fields. This is especially true for research conducted in Europe, because:

1. For historic reasons, ciliate molecular biology has prospered mostly in the US, which until now has been in the vanguard. Periodic exchanges and international conferences have stimulated European researchers and kept them apace, but this is not sufficient to make them the leading force in the field.

2. The European ciliate community has been recently strengthened by establishment of independent young researchers after post-doctoral training in the U.S. The research topics of both new groups are exclusively centred on epigenetic mechanisms during macronuclear development and each of the new research groups is supported by an ERC grant. Along with the unflagging efforts of different research groups in the past decades, the European ciliate community is now in good position to take the leading role for ciliate molecular research in the world, provided there is efficient coordination between the existing groups and the new forces.

3. In Europe, the excellent tradition in basic sciences has created great diversity in ciliate research. One significant advantage the European ciliate community holds is the equilibrium in developing different disciplines and model species. Among conventional ciliate models, *Paramecium* is best studied in Europe and has created its own scientific network recently mainly with national support from France. Knowledge and technologies needed for studying other important lineages of the same phylum, such as *Euplotes* and *Stylonychia*, are also prosperously developed in Europe. The new joining groups have brought in new ideas and competences on another two popular ciliate models, *Tetrahymena* and *Oxytricha*. Altogether it is clear that the European community is nowwell prepared for a highly interdisciplinary project supported by this COST Action.

Above all, establishment of a highly interactive network is expected to revolutionize knowledge and technology transfers within this community, to guide early-stage researchers and to unite experts of diverse disciplines, and finally, to bring the European ciliate research community to the leading position in the world.

B.3 Reasons for the Action

Establishing the first European ciliate network would be quite timely: with spectacular recent advances, ciliates are coming of age as model systems for the basic problems mentioned above, and this COST Action would be the best way to capitalize on the recent but fragile trend towards an increasing number of European groups and increasing scientific quality.

The primary benefits that may be expected are a better understanding of the mechanisms of genome evolution and of non-Mendelian inheritance, and of the relative contribution of these processes to environmental adaptation. As has been the case in the past, many of the discoveries made in ciliates are likely to be applicable to other eukaryotes, where they might be more difficult to detect or to study. For instance, a detailed understanding of the roles and mechanisms of action of meiosis-specific small RNAs in ciliates may shed light on the possible implication of piRNAs, with which they show intriguing similarities, in non-Mendelian inheritance and adaptation in metazoans.

Establishment of this research network is not only an urgent need from the ciliate community in Europe, but also a substantial contribution to the persistent development of the European scientific society. Exploration of the most important scientific questions in ciliate research, as well as in the forefront fields of modern biology, will surely add great value to basic research in Europe, and enhance the competitive strength of European research in the global environment.

B.4 Complementarity with other research programmes

There is no any current or planned European research project, except the ERC grants, that is significantly connected with this proposal. Most of the research activities carried out by the network participants received financial supports at national level. However, the Paramecium European Research Group (core teams from France, Italy, Germany and Poland) created under French CNRS auspices, will help structure the COST Action and in return benefit from the enlarged and highly stimulating environment of the COST Action. The Paramecium network is only financed for small meetings and workshops. Exchanges between labs in different countries have been possible in the past through ESF travel grants. However the ESF will no longer finance exchange of early-stage researchers, highlighting the enormous value of the COST Action for scientific collaborations, information exchange and training of early-stage researchers.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The aim of this Action is to establish a network of ciliatologists in order to strengthen and consolidate European research in this area aiming at deciphering the molecular mechanisms underlying epigenetics and non-Mendelian inheritance and environmental adaptation.

C.2 Secondary objectives

Secondary objectives of the Action are:

1. To demonstrate that ciliates are optimal model organisms to study epigenetic phenomena and the mechanisms of environmental adaptation, among other important biological fields;

2. To provide a platform for technology transfer within the European ciliate community;

3. To unite experts of diverse disciplines. The multidisciplinary Action will create opportunities to interact with/incorporate researchers from the fields of bioinformatics, population genetics and phylogeny, with whom most ciliatologists currently do not even have a chance to interact. Novel cutting-edge research domains are expected to be created through these interactions;

4. To guide early-stage researchers. The creation of a highly interactive network of "ciliatologists" will improve the quality of scientific production and the training of young researchers, including PhD students. Regular meetings will circumvent the current lack of opportunities for young researchers to expose their research and to obtain valuable feedback from specialists. Moreover, the action will enable young researchers to visit other laboratories and learn techniques through hands-on training.

C.3 How will the objectives be achieved?

Highly interactive European network of "ciliatologists" and exchange of information will be established. It will be achieved by development and strengthening of links between ciliate research institutes. Creation of a network platform to allow the transfer of knowledge among the members of the Action. The network will allow the joining of additional groups. The continuous interaction and exchange of results among the groups will be coordinated by a leader group that will be changed every year. Establishment of international databases. Members of the network already possess the resources and expertise for this purpose; witness the Paramecium DB model organism database and its wiki community. Moreover, the genomes of *Euplotes focardii*, an Antarctic ciliate species, and of several additional *Paramecium* species will soon be released.

The Action will promote knowledge advance and transfer. It will be achieved by Organization of mobility programs (at least two/year), which provide the opportunity to exchange researchers and students from the different groups of the Action for work and/or studies. Mobility programs are also intended to increase the number and the quality of joint publications.

Moreover, the Action will unite experts of diverse disciplines and training of early-stage researchers. It will be achieved by organising of meetings (two/year), among which the European conference on Ciliate Biology and the FASEB summer conferences on Molecular Biology of Ciliates, usually held in the US but planned in Greece in 2011. Organization of training workshops (at least one/year) focusing on genetics and cell biology of ciliates, with the involvement of field specialists.

C.4 Benefits of the Action

The primary benefit of the Action will be an increased visibility of the European research in the field. The creation of a "ciliatologists" network will facilitate the collaboration of European researchers, including new groups that are emerging with the support of the European Commission. At the scientific level, the Action will provide the tools and new concepts for a better understanding of the mechanisms of genome evolution and of non-Mendelian inheritance, and of the relative contribution of these processes in environmental adaptation of ciliate models.

C.5 Target groups/end users

All of the groups involved in the preparation of the proposal will be interested in the outcomes and will exploit the expected results. In addition, as has been the case in the past, many of the discoveries made in ciliates are likely to be applicable to other eukaryotes, where they might be more difficult to detect or to study. For instance, a detailed understanding of the roles and mechanisms of action of meiosis-specific small RNAs in ciliates may shed light on the possible implication of piRNAs, with which they show intriguing similarities, in non-Mendelian inheritance and adaptation in metazoans. More generally, the knowledge expected to be produced through the Action will likely be of use to other researchers in the fields of epigenetics, genome evolution, population genetics and environmental science.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

The main research tasks to be coordinated by the Action are the followings:

1. *Establishment of a long-lasting forum*. This task consists in the creation of an information network interconnecting all members of the action. This network will enable the continuous interaction and exchange of outcoming results and genomic data among the members of the groups. To exchange fragmented knowledge and technologies among European ciliatologists, this task of the Action also includes: a- the organization of regular meetings to train young scientists on basic and advanced methods on ciliate genetics, molecular and cell biology; b- the exchange of researchers between laboratories (STSMs) that will involve both young and senior researchers; c- organization of meetings that will allow the monitoring of the training of the young researchers and the progress of the Action;

2. *Developmentally regulated, alternative genome rearrangements showing non-Mendelian inheritance.* Although several lines of experimental evidence have indicated that epigenetic programming of developmentally regulated genome rearrangements in the zygote is mediated by long and short RNA molecules produced from the maternal somatic and germline genomes, the underlying molecular mechanisms are still poorly understood. The Action will try to identify and characterize genes and proteins involved in programmed genome rearrangements to better understand how the relevant RNA molecules are produced, how they interact with chromatin, how they induce chromatin modifications, and how the latter regulate genome rearrangements. In parallel, high-throughput sequencing will be used to extensively characterize the RNA molecules involved. Similarly, efforts will be made to understand the role of RNA molecules in the control of the copy numbers of individual macronuclear chromosomes. Since regulating alternative rearrangements and copy numbers of polyploid chromosomes can be an efficient way to quickly adapt to environmental changes, understanding the mechanism behind this regulation should provide us with tools to dissect the potential roles of non-Mendelian inheritance in environmental adaptation processes;

3. *Genetic variation of adaptive significance.* Natural selection leads to adaptations at multiple biological levels (molecular, cellular, physiological/integrative, and behavioural) as organisms respond to diverse environments. Temperature is one of the principal environmental variables that drive this adaptive evolution, particularly in ectotherms (organisms whose body temperatures conform to ambient temperature). At the molecular and cellular levels, small thermal changes cause profound alterations in enzyme activity, protein stability and transport, macromolecular assembly reactions, membrane fluidity, secretory processes, and neuronal transmission (Hochachka and Somero, 2002). Thus, polar marine ectotherms, most of which derive from temperate ancestors, evolved compensatory restructuring of their biomolecular systems to preserve appropriate biological activity as their habitats cooled. However, the mechanisms of adaptation to low temperature regimes remain poorly understood.

To understand the “first principles” of the psychrophilic (cold-loving) mode of life, members of the Action are studying the genome and the transcriptome of a psychrophilic ciliate, *Euplotes focardii*. The comparison of these sequences with those from *Euplotes* species adapted to different environments will help identify the molecular mechanisms responsible for long-term cold-adaptation, for example through the identification of new protein isoforms or epigenetic mechanisms;

4. *Linking non-Mendelian inheritance to environmental adaptation.* One of the central hypotheses which the Action is intended to test is that environmental changes can induce alternative DNA rearrangements and chromosome copy numbers that are subsequently inherited in sexual progeny. Combining the molecular knowledge and genetic tools expected to be obtained in the first subject and a solid environmental adaptation model expected to be established in the second subject will be essential to provide the first clear demonstration of environmental adaptation through non-Mendelian inheritance of epigenetically determined characters.

These topics are of interest to all of the participants in the network and allow the integration of new participants at different stages in the course of the Action.

This Action potentially unites 35 groups (14 have been already involved in this proposal) with a human potential of nearly 180 scientists, with more than 60% of early stage researchers. Participants will be from 9 COST countries, plus 6 non COST countries.

D.2 Scientific work plan, methods and means

Three working groups (WG) will be set up corresponding to the four tasks to be accomplished. These four WG are highly interacting since the experimental approaches 3 and 4 depend on the second scientific topic, and all of them will be linked by the first task.

WG 1: *Developmentally regulated, alternative genome rearrangements showing non-Mendelian inheritance.*

This WG has three tasks:

A. *Identification and characterization of genes/proteins involved in epigenetic programming of developmentally regulated genome rearrangements and in macronuclear chromosome copy number control*. Candidate-driven RNAi screens to identify novel genes involved in programmed genome rearrangements will be performed in the *Paramecium* system. In parallel, proteins interacting with the known players will be identified by affinity purification followed by mass-spectrometry analysis, and their functions will be analysed by reverse-genetics in *Tetrahymena*. Genes involved in the macronuclear chromosome copy number control will be identified and analysed by RNAi-based screening in Oxytricha/Stylonychia. The relevance of epigenetic markers such as DNA-methylation or chromatin modification for these processes will be analysed in Stylonychia;

B. *Establishment of viable genetic mutant models that alter genome rearrangements and/or their non-Mendelian inheritance.* As a complementary approach, forward genetic screens will be implemented in *Paramecium* using the mating-type determination system: this was recently shown to depend on a maternally inherited alternative rearrangement that is exquisitely sensitive to the slightest dysfunction of the meiosis-specific small RNA pathway ensuring non-Mendelian inheritance, which should allow the recovery of hypomorphic alleles of essential genes. Once genes/proteins are identified through plans A and B, the function of any homolog present in other ciliates, including *Euplotes focardii*, will be analysed by RNAi-based reverse genetics to test whether non-Mendelian inheritance plays any role in environmental adaptation processes such as cold adaptation;

C. *Characterization of long and short RNA molecules.* Long and short non-coding RNA molecules, proposed to regulate programmed genome rearrangements in a homology-dependent manner will be analysed by a next-generation sequencing technology in *Oxytricha*, *Paramecium* and *Tetrahymena* systems. Similarly, long non-coding RNAs involved in the copy number control of the polyploid macronuclear chromosomes will be analysed in *Oxytricha* and *Stylonychia*. To interpret their biological roles in those processes, RNAs from wild-type and mutants or RNAi knockdowns expected to be obtained through the plan A above will be compared.

WG 2: *Genetic variation of adaptive significance.*

A. By comparing the genome of Antarctic and mesophilic ciliates, it will be possible to identify new protein sequences unique to the Antarctic species. These sequences will be analysed to discover their function and their role in cold adaptation. For example, to determine if these new proteins are important for the survival of *E. focardii* cells at low temperatures, their translation will be blocked by the use of the RNA silencing technology, and survival examined. The evolutionary history of these proteins will be reconstructed by sequence comparison. This analysis is expected to unravel the molecular mechanisms responsible for long-term cold adaptation.

B. A deeper analysis of the larger nanochromosomes of *E. focardii* will help to unravel the intriguing possibility that they may contain multiple genes and perhaps be organized for co-regulation as in bacterial operons. The hypotrich species *Oxytricha fallax* and *O. trifallax* have been shown to generate two two-gene nanochromosomes by alternative processing of a micronuclear locus (Seegmiller et al., 1997) possibly a mechanism to ensure co-regulation of expression. *E. focardii* may also generate multigene nanochromosomes, where genes are grouped by function and/or by involvement in cold adaptation.

C. To unravel the molecular mechanisms responsible for a short-term thermal adaptation, the differential gene transcription in response to thermal stress will be analysed at whole transcriptomic level in cells of the psychrophilic and mesophilic *Euplotes* species incubated at different temperatures.

WG 3 : *Linkage of non-Mendelian inheritance to environmental adaptation.* The more relevant scientific innovativation of this Action will be to try to link non-Mendelian inheritance to environmental adaptation.

A. *Selection and identification of epigenetically inherited alternative genome rearrangements with adaptive potential.* Starting from large populations of genetically identical cells with independently developed macronuclei, genome rearrangement variants that allow faster growth under extreme conditions (e.g., high temperature, high salt concentration, etc) can be selected by applying these conditions for a large number of vegetative divisions, with periodic induction of sexual processes to generate new macronuclei. Beneficial alternative rearrangements that can be epigenetically transmitted to sexual progeny are expected to increase in frequency in each generation, allowing the progressive build-up of more efficient macronuclear genomes through combinatorial effects. After several cycles, isolated clones will be tested and compared with the original strain, and the mode of inheritance of any adaptive phenotype will be determined by genetic analysis. In the absence of mutagenesis, Mendelian mutants are not expected; for phenotypes showing non-Mendelian inheritance in crosses, the occurrence of alternative genome rearrangements or copy number variants will be identified by high-throughput sequencing of the whole macronuclear genome. The same scheme will also be applied after partial or complete knockdown of genes involved in the epigenetic regulation of genome rearrangements (for species in which these have already been identified) during macronuclear development, since this is known to generate a large diversity of alternative genome rearrangements.

B. *Environmental stress and modification of epigenetic programs.* Whatever the frequency of spontaneous alternative rearrangements with adaptive value to a particular condition, a key question is whether environmental stress in itself can affect the RNA-based mechanisms of epigenetic programming, resulting in an increased frequency of alternative genome rearrangements. This would be consistent with recent evidence that the environment can stably influence the establishment of the epigenome in other eukaryotes (Daxinger and Whitelaw 2010, Genome Res. 20:1623-1628); in Paramecium, it has been shown that temperature, for instance, can influence the determination of mating types (Sonneborn 1977, Ann. Rev. Genet. 11:349-367), now known to depend on alternative rearrangements. This question will be addressed by inducing sexual processes and macronuclear development in large populations of cells submitted to a variety of stressful conditions, and then comparing the frequency of selectable alternative rearrangements arising in these conditions and in normal conditions. If an increased frequency is detected, molecular studies will be conducted to determine what step, if any, of the known regulatory pathways (such as the meiosis-specific scnRNA pathways in Paramecium and Tetrahymena) are affected. Efforts will also be made to determine whether induced alternative rearrangements are random or perhaps biased towards beneficial forms, which could be the case if cells have evolved ready-to-use epigenetic switches to adapt to particular conditions.

E. ORGANISATION

E.1 Coordination and organisation

The main goal of this Action is the development of an innovative, highly interactive, dynamic European network of ciliatologists. The mini-symposium “Molecular Biology of Cilliates” held in Camerino, in September 2010, organized by the proposer of the Action, has laid the basis for a European network, open to researchers worldwide. The research topic will focus on the molecular biology of ciliates, with particular attention on epigenetic phenomena. Their core funding is assured by different national and international programs; however COST is the ideal framework for the realisation of ‘horizontal cross-links’ that provide added value for each of the subdisciplines.

The “horizontal cross-links’ will be represented pragmatically by an informatic network allowing each group to publish their results and that will allow to share outcomes, databases and websites. The set-up of shared internet resources will be valuable for the experimental groups involved in the Action, allowing the exchange of technical information on the design and implementation of systems. A Management Committee responsible for monitoring the advancement of the Action will be formed. The Management Committee will be coordinated by a leader group which will be changed every year. The members of the Committee will be elected during business meetings to be held at the annual scientific meetings. The members of the Committee will be in contact by e-mail and by the use of the informatic network. Possible problems will be solved both using the informatic network, and face-to-face at meetings, workshops and schools organized by the Action. The Management Committee will be also responsible for the continuous update and maintenance of the informatic network.

To achieve the best coordination of the participating research teams, exchange on all levels (PhD students, postdocs, senior scientists) will be facilitated between research groups. This will include annual workshops for all the participating research groups. In addition to the exchange between research groups, a summer school component will contribute to the training of younger researchers. The thus established personal contacts and connections will form the basis for successful future collaborations. Mutual visits of (young) researchers for periods between a few weeks to 3 months or 6 months in case of Early Stage Researchers (ESR) will allow efficient collaboration on certain subtopics and will strengthen the ties between the member groups. Knowledge transfer and training of young scientists will also be achieved by the organisation of COST Action meetings in conjunction witch the European conference on Ciliate Biology and the FASEB summer conferences on Molecular Biology of Ciliates, usually held in the US but planned in Greece in 2011.

The Management Committee will be in charge of piloting and evaluating the ongoing Action with respect to the milestones defined in section D.2. It will also be responsible for the dissemination of results throughout the whole community, and will be in charge of boosting visibility of the research on ciliates in Europe. Moreover, it will prepare and organise the dissemination of results to a wider scientific community, and in particular to interested new groups. The steering committee will in particular encourage the emergence of novel scientific topics and applications.

Evaluation will concern all of the activities cited earlier in this section, in terms of their efficiency and scientific output. This will be scheduled twice a year, in order to encourage or eventually re-orientate certain topics and to maintain an overall equilibrium of the research carried out, in view of the defined objectives.

E.2 Working Groups

Three working groups will be set up, each dealing with a different topic (see D.1 and D.2). The coordinators of each of the WGs will (a) coordinate the activities within their WG in order to meet the objectives that are defined in the scientific program; (b) stimulate and foster the set-up of joint research (e.g. making use of STSMs); (c) promote the writing of common publications; (d) report the WG progress to MC.

Meetings of the WGs will be organized at least once a year at different partner locations. It is planned to hold two to three day meetings for each WG in order to maximise the exchange of ideas. This will encourage the collaboration between scientists and institutes, stimulate the planning of joint experimental work and address WG specific topics.

E.3 Liaison and interaction with other research programmes

The Paramecium European Research Group (core teams from France, Italy, Germany and Poland) created under French CNRS auspices, will help structure the COST Action and in return benefit from the enlarged and highly stimulating environment of the COST Action. The Paramecium network is only financed for small meetings and workshops. Exchanges between labs in different countries have been possible in the past through ESF travel grants. However the ESF will no longer finance exchange of early-stage researchers, highlighting the enormous value of the COST Action for scientific collaborations, information exchange and training of early-stage researchers.

Liaison and interaction will be also provided by the recently approved project FP7-PEOPLE-2009-IRSES "CIliates as NAtural Reservoir of potentially PATHOgenic BACTERia: an ecological, functional and evolutionary genomic investigation". This project (the coordinator has already expressed interest in the participation to this COST Action) has the aim to perform a complete ecological, functional and evolutionary genomic investigation on symbiotic bacteria retrieved from ciliates showing phylogenetic affinities to pathogenic ones. The adaptive evolution of ciliate hosts and symbiotic bacteria may be correlated to epigenetic mechanisms discovered by the research activities of the participants to this COST Action

E.4 Gender balance and involvement of Early-Stage Researchers

At present the members of the network are gender balanced (almost 40% of the participants are women) and it is intended to maintain this balance. In this context, it is worth noting that the proposer is a woman. A particular effort will be made to encourage female scientists throughout this Action, by implying them preferentially in all responsibility tasks. As expressed in the European Charter for Researchers and the Code of Conduct for the Recruitment of Researchers (C&C), the Action will promote a strict equal opportunity policy and encourage female researchers to apply for positions in the field. The Action will also particularly insist on attracting women as young researchers, e.g., through the introduction of special contributions that guarantees the payment of their salary during maternity leave. One general objective of the Action will be also to implement the C&C principles in the involved institutions for what concerns the activities of the Action and possibly using the Human Resources Strategy for Researchers (according to the web site http://ec.europa.eu/euraxess/index.cfm/rights/strategy4Researcher).

Early-Stage Researchers account for more than 60% of the scientific personnel involved in this Action and will hence particularly benefit from its activities. The involvement includes both training and conducting the research. Early-stage researchers are supposed to spend periods of time, from a few weeks to six months, in the laboratories of the participants of the Action. Each WG will elect a “junior” representative which will be a privileged discussion partner to the WG coordinator concerning any issue specific to early-stage researchers. These four representatives will be part of the management committee.

They will also be in charge of organizing workshops and summer schools to allow the discussion of specific questions, in the creation and maintenance of the informatic network, in which each group will publish its own results and will share databases, will allow continuous interaction and exchange of outcomes among the early-stage researchers.

F. TIMETABLE

The duration of the proposed Action is estimated to be four years. This time is necessary to create an efficient interaction between the groups and build lasting links to allow working groups to organize and work efficiently. The proposed timetable may be reformulated if this appears to be necessary during the running of the Action.

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<tr><td></td><td colspan="4">Tasks</td></tr>
<tr><td></td><td>Coordination</td><td>Non-Mendelian inheritance.</td><td>environmental adaptation</td><td>non-Mendelian inheritance and environmental adaptation</td></tr>
<tr><td rowspan="4">Year 1</td><td colspan="4">“COST Action” kick-off meeting: election of the MC and set-up of working groups</td></tr>
<tr><td></td><td>WG1 meeting</td><td>WG2 meeting</td><td>WG3 meeting</td></tr>
<tr><td>Creation of the platform</td><td>Set-up of the first experiments</td><td>Set-up of the first experiments</td><td>Set-up of the first experiments</td></tr>
<tr><td colspan="4">Training school for Early-Stage Researchers</td></tr>
<tr><td rowspan="5">Year 2</td><td colspan="4">STSMs</td></tr>
<tr><td colspan="4">MC meeting: scientific evaluation, orientation and schedule.</td></tr>
<tr><td></td><td>WG1 meeting</td><td>WG2 meeting</td><td>WG3 meeting</td></tr>
<tr><td colspan="4">Common workshops with publication of special issue</td></tr>
<tr><td colspan="4">STSMs</td></tr>
<tr><td>Up-dating of the platform. Build additional links</td><td>Task A</td><td>Task A</td><td>Task A</td></tr>
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Year 3	MC meeting: scientific evaluation, orientation and schedule			
		WG1 meeting	WG2 meeting	WG3 meeting
	COST International Conference			
	Training school for early-stage researchers			
	Up-dating of the platform. Build additional links	Task B	Task B	Task A and B
Year 4	MC meeting: scientific evaluation, orientation and schedule			
		WG1 meeting	WG2 meeting	WG3 meeting
	Common workshops with publication of special issue			
	Up-dating of the platform	Task C	Task C	Task C
	Final report and joint review publication			

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, CH, DE, ES, FR, HR, IT, PL, PT,UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 40 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?

The research groups that participate to the Action are the primary beneficiaries. The research conducted through the collaborative Action probably will provide added value to the outcomes and will improve the quality of the publications. The wider protozoologist community and non-participating research groups in the field will benefit from the results published in the scientific literature and from the possibility to take part in the meetings and workshops periodically organised as part of the Action, and through collaborative projects with member groups.

As has been the case in the past, many of the discoveries made in ciliates are likely to be applicable to metazoans, and the expected results will be of considerable value also for neighbouring research communities such as those involved in cellular biology, physiology, medicine, environmental biomonitoring and evolution. For instance, some epigenetic phenomena occur as part of cell development, including mechanisms for balancing the expression of genes on sex chromosomes whose dosage differs in males and females, or mechanisms that limit expression of genes to maternal or paternal alleles. Other epigenetic phenomena are strongly implicated in human diseases, including cancer. The analysis of the relationship between non-Mendelian inheritance and environmental adaptation may be applied to a variety of environmental influences, including diet or environmental toxins, which can affect gene-expression levels for multiple generations.

Moreover, Master-level and PhD students will directly benefit through the teaching and career development activities organised within the framework of the Action. They will have the support of “mentors” represented by international experts in the fields who are members of the network. The creation of an informatic network will allow continuous interaction and exchange of outcomes among early-stage researchers. Organization of mobility programs, meetings and training workshops will provide the opportunity for researchers and students to exchange and improve their “know how” and to increase the number and the quality of joint publications.

H.2 What?

The Action's dissemination methods rely on the following strategies:

1. Periodically organised workshops and meetings will enable the exchange of knowledge and expertise among the participating groups and facilitate the planning of joint projects and publications. Participation in the workshops will also be promoted among non-members of the Action, thereby establishing and consolidating links with the worldwide ciliatologist community;
2. Publication of the results achieved within the Action's activities in scientific journals and presented at national, European and international conferences will make them available to the wider scientific community;
3. The creation of the informatic network will represent the essential part of the dissemination strategy. It will contain knowledge databases and newsgroup, thereby acting as an information portal for all members. The website will also promote the activities and results generated through the collaborative efforts to the wider scientific community and facilitate the communication with the general public;
4. The training schools organised in conjunction with the Action's workshops are aimed at training students and postdoctoral researchers in the basic and advanced techniques used in molecular biology of ciliates;
5. In all possible cases, the activities of this Action will be diffused by the use of the Euraxess portal to provide information and opportunities for international collaboration.

H.3 How?

To make our results available to the wider community and to encourage a dialogue with all interested parties, particular efforts will be made to include ciliates as model organism in many workshops and courses organised by EMBO/EMBL/EBI. Moreover, efforts will be made to participate in congresses not specifically focused on ciliates. For example, the participation of the proposer to the "FASEB Summer Research Conferences: The Biology of Cilia and Flagella" allowed the inclusion of ciliates as models for studying this topic.

In a general way, this Action will strengthen the interactions with the neighbouring disciplines and topics through talks and conference invitations in order to disseminate results directly to interested colleagues.

Other means will be the publication of proceedings after each congress planned during the Action. Tutorials given during the training schools will be published in a special edition, and will be downloadable on the internal website for all groups participating in the Action.

Additional dissemination methods will be the organization of seminars, open days, and lectures in high schools, especially to promote ciliates as model organism in biology. Moreover, members of the Action will participate in local events, for example “La Notte della Ricerca” in Italy, or the French “Fête de la Science”.