CNRS CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

Sciences du Vivant - Environnement et Développement durable

CONFÉRENCES JACQUES-MONOD

Jacques-Monod conference
Roscoff (France), May 3-7, 2006

MULTIPLE FUNCTIONS OF RNA IN GENE REGULATION
FONCTIONS MULTIPLES DE L'ARN DANS LE CONTRÔLE GENETIQUE

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Report on the conference
Rapport de la conférence

The CNRS-Jacques Monod conference “Multiple roles of RNA in gene regulation” was held in Roscoff, a charming village in Brittany, May 3-7 2006. The main idea behind this meeting was to bring together a distinguished group of scientists working on various aspects of regulatory RNAs, to review and strengthen this rapidly developing research field, and to emphasize the versatility of RNA structure and function. This conference highlighted new features that have emerged over the last few years on the RNA-based regulatory networks in eukaryotes – animals and plants –, and in prokaryotes. We realized very quickly that the selected topics were of high interest to the scientific community. Consequently, the conference was soon oversubscribed. We accepted 88 participants from a total of 79 different laboratories and, sadly, had to reject 110 applicants. Selection of presenters was not an easy task, and many interesting projects had to be excluded due to space limitation at the conference site. All in all, we arrived at a balanced and high quality selection of participants from 17 different countries (France, Germany, Greece, Italy, Spain, Sweden, The Netherlands, Denmark, Austria, England, USA, Japan, Russia, Mexico, Israel, Taiwan, and Switzerland).

This conference featured many of the best specialists in the field concerned. This high level line-up was highly appreciated by the participants. We had, early on, made a decision to select a sizeable fraction of young researchers (30 % of the participants) so that they would get the benefit of meeting and interacting with leaders in the field – an issue of importance for the new generation of promising scientists. The relatively small scale of the meeting, the intimate and peaceful atmosphere, and the excellent facilities provided by the CNRS in Roscoff, have stimulated many in-depth discussions and possibly initiated collaborations. Much of this must be credited to Mrs Dominique Lidoreau and the personnel of the Roscoff center that took care of the practical organization (administration, lodging, poster room, computer facilities…). Particularly valuable has been the fact that the meeting has brought together scientists from widely different cultures and scientific backgrounds, and in particular specialists in both eukaryotic and prokaryotic biological systems. We strongly feel that the artificial divide between different subject niches often is counterproductive. Thus, we favor diversity and interdisciplinary approaches, and such a broad coverage was certainly appreciated and considered to be an 'added value' to this conference.

Along these lines, the organizers chose a balance of contributions dealing with eukaryotic, prokaryotic and viral systems. A total of 28 speakers were invited (30 min talks), and 21 oral communications (15 min talks) were selected based on abstracts submitted by other participants. We also organized two poster sessions (2 x 30 posters) of at least two hours each. There was ample time and opportunity for questions and discussions, and similarly at the poster sessions. Both platform talks and poster sessions were highly appreciated. Even though the schedule was rather extensive, all participants had the opportunity to both deepen and widen their knowledge, and – judged by responses conveyed to the organizers – enjoyed the meeting greatly.

Scientific report

The role of non-coding RNAs in regulation of gene expression and genome stability has become a topic of soaring interest. In part this was fueled by the recent discovery that non-coding RNAs are much more common than previously believed, and evidence suggests fundamental roles for these RNA in all kingdoms of life. In particular, the discovery of RNA interference, and of miRNAs, has opened our eyes to the fundamental roles that such RNAs play in genome surveillance, developmental control, and biotechnology/ biomedicine. Meanwhile, many new reports show that the structure of mRNA is a key element in the regulation, by acting directly as a sensor (riboswitches induced either by environmental cues or small molecules) or by presenting specific binding sites for *trans*-acting factors (regulatory proteins or small non-coding RNAs). All central processes of gene expression, from mRNA biogenesis to translation and degradation, revolve around messenger ribonucleoprotein complexes (mRNPs). Recent large-scale characterization of several regulatory RNA-binding protein targets revealed that these proteins co-regulate mRNAs which are functionally related. An exciting parallel exists here between eukaryotes and prokaryotes – in spite of the use of (sometimes) different mechanistic solutions, regulatory RNAs may help out to promote similar crosstalk. The different topics presented during the meeting gave some insights in the connections between the different levels of regulation, and of the integration of these RNA-based regulatory networks into the cellular context.

The meeting started with two introductory lectures on the topics of RNA-evolution, followed by six sessions covering four main different aspects of the regulatory RNAs as summarize below.

- - RNA, epigenetic control & transcriptional control
- - Regulatory functions of miRNAs, RNAi & defense mechanisms,
- - snoRNAs in archae, sRNA and riboswitches in prokaryotes
- - mRNP in post-transcriptional control

Introduction

E. WESTHOF illustrated how RNA structure evolved to give rise to the different functions of today's RNA molecules. The unique three-dimensional structures adopted by these RNAs determine their activity. The resolution of the structure of the ribosomal RNAs pointed to the existence of redundant structural motifs that were subsequently found in other RNAs such as snRNA (C. BRANLANT) and in regulatory regions of mRNAs (M. SPRINGER, R. BREAKER). Certain structural motifs in RNA are essential for folding and stability. Using three-dimensional RNA building blocks, nanometer-scale structures with increasing complexity were obtained recently. E. WESTHOF emphasized the modular and hierarchical characteristics of RNA folding by showing that small RNA structural motifs can code the precise topology of large molecular architectures. Other structural motifs provide specific interaction sites for proteins. In some cases, the assembly of large mRNP/RNP complexes require a dynamic series of RNA structural rearrangements to arrive at an active complex. Ligand-induced conformational changes in segments of mRNAs (riboswitches) are now known to be at the heart of many events that activate or inhibit gene expression.

Before the sessions focusing on the eukaryotic regulatory RNAs, B. BASS presented an

overview on the dsRNA-mediated pathways in *C. elegans*. DsRNA is abundantly expressed in metazoan genomes: repetitive sequences in introns or untranslated regions form intramolecular structures, and antisense transcripts pair with mRNA to form intermolecular structures. B. BASS in particular presented a very suggestive link between RNA editing and RNA interference (RNAi), two processes previously believed to be unconnected. Adenosine deaminases that act on RNA (ADARs) are RNA-editing enzymes that deaminate adenosines to create inosines in double-stranded RNA (dsRNA). ADARs are not required for RNAi but play a role in regulating whether or not a dsRNA enters the RNAi pathway.

RNA & epigenetic control & transcriptional control – *ARN, contrôle épigénétique & transcriptionnel*

Rather unexpectedly, RNA was recently shown to play a direct role in epigenetic gene silencing (heritable changes in gene expression without changes in the DNA sequence), and a diversity of mechanisms were presented during this session (K. EKWALL, J. CAVAILLE, DP. BARLOW, MC. YAO). Mammalian genomic imprinting is one of the epigenetic gene regulatory mechanisms that results in parental-specific gene expression of a small number of genes in diploid somatic cells. Many imprinted genes are functionally grouped such that imprinted expression of several genes is regulated by one long-range imprint control element. Interestingly, imprinted non-coding RNAs are often expressed from the parental chromosome that carries the silent allele of an imprinted gene. D. BARLOW has demonstrated a silencing function for the long non-coding RNA Air in an imprinted gene cluster, on mouse chromosome 17, that contains three maternally expressed protein-coding genes and the paternally expressed non-coding Air RNA. This long RNA escapes splicing, and nuclear export, and is rather unstable. J. CAVAILLE has recently identified a novel long nuclear-retained poly(A) RNA, Bsr, which contains many imprinted non-coding RNA genes whose functions are still unknown. These small noncoding RNA genes belong to the C/D box snoRNA and microRNA gene families. The functional and evolutionary significance of an association between C/D box snoRNA genes, microRNA genes and genomic imprinting is still poorly understood.

Related to this latter point, transcriptional silencing in fission yeast requires several core components of the RNAi machinery. The recently discovered RNA-induced initiation of transcriptional gene silencing (RITS) complex, which contains siRNAs, an Argonaute protein and a putative RNA-directed RNA polymerase (RdRP), is required for heterochromatic silencing. At the mating-type locus, RITS is recruited to the centromere-homologous repeat cenH in a Dicer-dependent manner. Furthermore, the RNAi machinery operates *in cis* as a stable component of heterochromatic domains with RITS tethered to silenced loci by methylation of histone H3 at Lys9. This promotes the processing of transcripts and generates additional siRNAs for heterochromatin maintenance. K. EKWALL showed the involvement of the RNA polymerase II subunit Rpb7 in siRNA-directed heterochromatin formation at centromers. In Tetrahymena (MC YAO) and in Paramecium (S. DUHARCOURT), the development of a new somatic macronucleus after sexual events involves extensive rearrangements of the germ line genome. MC YAO proposed that an RNAi-related mechanism is instrumental in targeting of these germline-limited sequences for chromatin modification and subsequent DNA rearrangement. This RNA-guided DNA deletion is also used to eliminate foreign genes since an inserted *E. coli* gene was selectively deleted during differenciation.

Rather recently, several non-coding RNAs have been shown to control transcription by modulating the activity of proteins involved in transcription. This is the case for U1 snRNA, which associates with TFIIH and regulates transcriptional initiation (A. AKOULITCHEV), and 7SK snRNA, which inhibits the activity of the positive transcription elongation factor b (P-TEFb) (O. BENSAUDE). Mammalian cell extracts contain an inactive P-TEFb complex composed of four components: CDK9, cyclin T, the 7SK snRNA and the MAQ1/HEXIM1 protein. Binding of the 7SK snRNA converts the HEXIM1 protein into a P-TEFb (CDK9/cyclin T) inhibitor. Other RNAs both in eukaryotes (B2 RNA) and prokaryotes (6S RNA) affect the initiation of transcription through a direct interaction with RNA polymerase. The structure of an RNA polymerase II-RNA inhibitor complex was presented (P. CRAMER). It reveals that the RNA inhibitor occupies a novel site that overlaps with the binding site for nucleic acids in the elongation complex. A model was presented in which the RNA inhibitor prevented the downstream DNA duplex and the template single-strand from entering the cleft after DNA melting, and thus interfered with open complex formation.
Other evidence indicates that the nascent mRNA can regulate termination of transcription. As an example, a regulatory element was discovered located upstream of the *glmS* gene in Gram-positive bacteria, which functions as a metabolite-dependent ribozyme in response to glucosamine-6-phosphate (R. BREAKER). Thus ribozyme switches may have functioned as metabolite sensors in primitive organisms, suggesting that modern cells retain some of these ancient genetic control systems.

Regulatory functions of miRNAs, RNAi, defense mechanisms, viral RNA and behavior – *Les fonctions régulatrices des miARN, ARNi, mécanismes de défense, ARN viral et conséquences*

Gene discovery has been biased towards mRNA and proteins for a long time. A new class of tiny regulatory RNAs (miRNA) has been discovered in all metazoa (≈100 different miRNA genes in *Drosophila* and *C. elegans* alone, and >250 miRNA genes in vertebrate genomes). miRNA genes are expressed under the control of their own promoters but are often arranged in clusters, suggesting them to be co-regulated. Maturation of the miRNA is a step-wise process that involves two double-strand-specific, RNAse III-like, enzymes.

While only a few of them have known functions, current knowledge suggests them to be important for the control of animal development and physiology (V. AMBROS, S. HAMMOND, O. VOINNET, N. BAUMBERGER). Mis-expression of miRNA can also be associated with human disease. S. HAMMOND had identified an oncogenic cluster of conserved miRNAs (oncomiR-1) that is overexpressed in a wide range of tumor-derived cell lines and primary tumors. Ectopic expression of oncomiR-1 accelerates tumorigenesis in a mouse model for Burkitt's lymphoma. V. AMBROS compared the roles of conserved let-7 miRNA family genes in *C. elegans* and *Drosophila melanogaster* using genetic approaches. While in *C. elegans* three of the miRNAs of the let-7 cluster coordinately regulate the post-transcriptional expression of the *hbl-1* gene encoding a transcription factor involved in L2 and L3 developmental events, this cluster of miRNAs in *D. melanogaster* regulates systems that govern adult behavior and fertility.

MiRNAs can work essentially via two modes of action. In plants, miRNAs base-pair with mRNA targets by complete or nearly complete complementary, and induce degradation of the target mRNA involving the RNAi machinery (N. BAUMBERGER, O. VOINNET). In

animals, miRNAs mostly exhibit partial complementarity with the target mRNAs, and recent observations strongly suggest that they repress translation at the initiation step (W. FILIPOWICZ, H. GROSSHANS). Effects of miRNAs on translation can be mimicked in human HeLa cells by the miRNA-independent tethering of Ago proteins to the 3'-UTR of a reporter mRNA (W. FILIPOWICZ). Inhibition of protein synthesis occurs without a change in the reporter mRNA level and is dependent on the number of the hairpins tethering hAgo2 to the 3'-UTR. These findings indicate that the primary function of miRNAs is to guide their associated proteins to the mRNA. W. FILIPOWICZ showed that repressed mRNAs accumulate in cytoplasmic processing bodies (P-bodies) for either storage or degradation. Interestingly. I. BEHM-ANSMANT demonstrates that the P-body component GW182 links the miRNA pathway to mRNA degradation by interacting with AGO1, targeting bound transcripts for decay. Repressed mRNA can exit from the P-bodies to re-enter translation when human cells are subjected to stress (W. FILIPOWICZ). This derepression is mediated by the RNA-binding protein HuR which binds to AU-rich sequences in 3'UTRs and alters the potential of miRNAs to repress gene expression.

RNA-mediated gene silencing was first demonstrated in plants. At present, three different mechanisms are known to account for silencing (N. BAUMBERGER, O. VOINNET, T. ELMAYAN): a defense mechanism against viruses, regulation of gene expression at the post-transcriptional level (PTGS), and induction of transcriptional silencing through DNA methylation (TGS). In plants, silencing signals can be amplified and transmitted between cells, and even regulated by a feedback mechanism. A tremendous amount of work in plants has been aimed at studies of the diversity of RNA silencing mechanisms and the relationships with siRNA and miRNA pathways. The emerging picture is complex since, even if the different pathways can be genetically and biochemically differentiated, they can also intersect and interact (N. BAUMBERGER, O. VOINNET). As expected from an evolutionary "Red Queen" scenario, viruses have consequently evolved various strategies to counteract this defense mechanism (N. BAUMBERGER, O. VOINNET), several of which have provided excellent tools to interfere with distinct steps in RNA-mediated silencing. Interestingly, in the ameoba *Dictyostelium*, two proteins, Eri1 and HelF, were shown to counteract the action of siRNAs and related effectors: Eri-1 inactivates siRNAs by cleaving off their essential 3' overhangs, and HelF belongs to the RNA helicase family that serves as a negative regulator of RNAi but not of antisense RNA regulation (W. NELLEN). A useful information provided by G. SCZAKIEL is that phosphorothioate-derived oligonucleotides can stimulate the physical cellular uptake of siRNA *in trans* in human cells for target-specific inhibition.

Virus research has pioneered many discoveries of much more general mechanisms. This includes mechanisms of translational control (initiation via an internal ribosomal entry site: IRES in picornavirus, R. JACKSON) and the host response via silencing (O. VOINNET). Still poorly understood are the viroids, which can be regarded the simplest forms of viruses of the non-coding RNA world (F. Di SERIO). These non-protein coding, circular and single-stranded RNAs rely for replication and propagation almost entirely on the pre-existing machinery of their host. As a consequence, regulation of host gene expression becomes impaired leading to the emergence of disease. An entirely unanticipated link between genes and behavior has emerged from the study of social insects. The presence of a new picornavirus (RNA virus) in the brains of attacker honeybees might be associated with aggressive behavior (T. FUJIYUKI).

snoRNAs in archae, sRNAs, and riboswitches in bacteria – *snoARNs dans les archae, sARNs, et riboswitch dans les bactéries*

Before the discoveries in eukaryotes, the importance of small regulatory RNAs in bacteria was suggested already in the 80's, in studies of plasmid-encoded antisense RNAs. Today numerous new non-coding RNAs (sRNAs) have been discovered in *E. coli* and related bacteria (today: > 80 in *E. coli*). In contrast to miRNAs, these sRNAs are diverse in size and structure. The emerging roles, that many of sRNAs play as regulators, are actively pursued (G. WAGNER,). It appears that many of them are antisense RNAs that target mRNAs, either through activation or inhibition. Other RNAs sequester specific proteins that in turn have regulatory roles. In general, the sRNAs are only expressed under particular conditions, and thus may help the cell to rapidly adapt growth to environmental cues or stress signals (G. WAGNER). As an example, IstR1 inhibits the translation of *tisB* mRNA encoded a toxin, in the SOS response to DNA damage. The inhibition of *tisB* translation occurs at a long distance and involves most probably blockage of the ribosome stand-by at a site far upstream of the *tisB* translation initiation region. Many of the sRNAs target mRNAs encoding membrane proteins, suggesting that sRNAs induce a remodeling of the bacterial surface composition in response to environmental conditions (G. WAGNER, M. GUILLIER, H. AIBA). A global regulatory protein, the Sm-like Hfq protein, assists several of the sRNAs that target mRNAs. In many cases, sRNA-mediated translational repression is associated with rapid decay of the repressed mRNA. It was proposed by H. AIBA that mRNA degradation is mediated by a ribonucleoprotein complex consisting of the sRNA associated with Hfq and the ribonuclease E.

There is also growing evidence that RNA is involved in regulation of virulence in many bacteria such as *Vibrio cholerae* (B. BASSLER) or *Staphylococcus aureus* (P. ROMBY) and *Listeria monocytogenes* (P. COSSART). In *V. cholerae*, a class of new RNAs appears to affect a key mechanism – quorum sensing – by targeting the expression of a transcription factor. In *S. aureus*, a strikingly versatile RNA, RNAIII, is a master regulator of virulence. It carries three functional units within the same molecule – a reading frame for a hemolysin, an activator antisense segment, and an inhibitory antisense segment which targets a class of mRNAs that encode adhesins and a transcription factor (P. ROMBY).

Work in recent years has shown that mRNAs can directly sense environmental cues (temperature induces a conformational change in the mRNA encoding a transcription factor that regulates virulence in *L. monocytogenes*, P. COSSART), or small metabolites such as vitamin B12, nucleobases, and amino acids by « riboswitch mechanisms » (R. BREAKER). Riboswitches are domains within the non-coding leaders of some mRNAs which carry the potential to fold into mutually exclusive structures. They serve as metabolite-sensing switches; metabolite binding causes allosteric changes in the mRNA that change gene expression – by inducing transcription termination or translation initiation. The discovery of a riboswitch that has ribozyme activity (R. BREAKER), and the inference that eukaryotes might use riboswitches for splicing control, hint at the potential for far greater diversity for riboswitch function in « ancient » and « modern » organisms.

Archaea were also recently shown to contain non coding RNAs similar to the eukaryal H/ACA snoRNAs that guide U to Y conversions in ribosomal RNAs. A computer analysis identified 45 new putative H/ACA snoRNA genes (C. BRANLANT). Using *in vitro* reconstitution a fully active sRNP particle was reconstituted with L7Ae protein which

facilitates the folding of the RNA and aCBF5, the RNA modified enzyme which recruited aNOP10 protein for its activity. The structure of L7Ae protein and of the aCBF5-aNOP10 complex were solved at high resolution, providing the basis for the identification of residues that are important for the assembly of the sRNP and for its activity (C. BRANLANT).

Mechanistic aspects of translational regulation – *Aspect mécanistique du contrôle traductionnel*

The importance of mRNA structure in regulatory mechanisms was highlighted in bacteria and in eukaryotes. Affecting mRNA structures, and the accessibility of structure elements and modules, is often critical for translational control both in prokaryotes and eukaryotes. Whereas classical cap-dependent translation is considered the standard control mechanism in eukaryotes, escape from this mode of regulation can be achieved by internal ribosome entry (A.C. PRATS, A. WILLIS), shunting (T. HOHN), polycistronic translation and other mechanisms (reprogramming of the ribosome). Even though, e.g., internal ribosome entry had mainly been studied in viruses, current work provides more and more insights into the use of this mechanism by cellular mRNAs (AC PRATS, A. WILLIS). For instance, some results indicate that an IRES – in conjunction with other levels of gene control – can promote significantly different protein output under different physiological conditions. Structural features and protein factors that bind to the viral IRES elements have been presented and discussed during the conference.

mRNP in post-transcriptional regulation – *mRNP et régulation post-transcriptionnelle*

Eukaryotes encode many more RNA binding proteins than prokaryotes. This has been taken to indicate that RNA-binding proteins provide the means to couple transcription and translation in eukaryotes. This would occur through protein-RNA interactions at each step: from transcription to RNA splicing, export, translation and stability. Such links between different steps are mediated by protein–protein interactions between specific RNP, components of the transcription, RNA processing and export machineries. These observations have been interpreted as evidence for molecular coupling of multiple processes and for RNA quality control (D. AUBOEUF, D. LIBRI, O. MÜHLEMANN, E. BERTRAND). D. AUBOEUF showed that a number of transcriptional coregulators are involved in mRNA maturation and participate in the export of their target gene products, thus allowing for coordinate control of the synthesis, maturation and fate of their target mRNAs. Cells have also evolved quality control mechanisms that rapidly recognize and eliminate aberrant products in a process termed RNA surveillance (Nonsense mediated decay, O. MÜLHEMANN). D. LIBRI described a novel class of cryptic unstable transcripts. These RNAs are distinguished from normal mRNA through a new and dedicated pathway of termination that triggers degradation instead of productive polyadenylation and export. Powerful strategies have now been developed to follow the movement of a defined mRNP in living cells, and to follow the intra-nuclear RNA transport with nucleo-cytoplasmic trafficking (E. BERTRAND).

Recently, RNA-binding proteins were shown to regulate multiple mRNAs in concert in order to orchestrate the final outcome. The concept of the post-transcriptional regulon states that mRNAs are utilized in multiple combinations in order to garner complexity from a relatively modest number of genes (JD. KEENE). This is the case of Nova and FMRP proteins (JD. KEENE), which bind to a subset of mRNAs that encode proteins involved in neuronal synapses, of EDEN-BP protein (B. OSBORNE), which regulates different mRNAs involved in *Xenopus* somatic segmentation, of CPEB which represses translation of CPE-containing mRNAs in *Xenopus* oocytes (N. STANDARD), and of ZBP (Zip code binding protein, S. HÜTTELMAIER). These ZBP proteins modulate the cytoplasmic fate of specific target transcripts during development and carcinogenesis. In stressed cells, ZBP protein prevents premature degradation of target transcripts, most probably by interference of mRNA sorting to processing bodies. These proteins bind to their own mRNA and inhibit translation by different mechanisms; this is the case for ribosomal protein L20 (M. SPRINGER). In this system, the protein recognizes a defined RNA motif in the leader region of its own mRNA, which mimics the natural substrate of the regulatory protein. Protein L20 plays the role of a chaperone by aiding the formation of an inhibitory structure within the mRNA.

This allowed J. KEENE to discuss an interesting concept, the so-called « post-transcriptional operon ». This model is based on the organization of genetic information at the level of eukaryotic mRNAs; RNA binding proteins interact with groups of monocistronic mRNAs that can be regulated together to provide a collective functional outcome. Such coordinated events include mRNA decay as well as mRNA translation, export or localization of discrete classes of transcripts. It was discussed whether such a concept may also apply for miRNAs, since for instance several of them target different mRNAs involved in the temporal development of *C. elegans* (V. AMBROS). Such a model could provide a rationale for how relatively few mammalian genes can be used in multiple functional combinations to coordinate the expression of complex genetic traits.

Conclusion

This “regulatory RNA” field is presently exploding, and exciting new developments are expected to emerge in the next years. During this meeting we were able to compare the function and the mechanism of action of regulatory RNAs across kingdoms.

This conference has been regarded as very successful by the participants, and the lack of any negative feedback certainly was encouraging. We received strong support by the participants (during the meeting but also after the meeting by receiving numerous e-mails) to further pursue the Jacques Monod conference in the field of post-transcriptional control, and obtained strong approval for the designated next president Prof. G. Wagner (Uppsala University, Sweden).

Although other conferences on RNAi, on posttranscriptional control, etc..., exist, none of these covers the diversity of topics that the Jacques-Monod conferences on posttranscriptional control have covered, from earlier conference to the one in Roscoff in 2006. This format is highly unique and much appreciated. Thus, a continuation of the series should by all means be guarantied.