Grant Agreement number:

Project acronym: OncoMiRNA-Biogenesis

Project title: Biogenesis of Oncogenic MicroRNAs: from the structure of the microRNA processing complexes to the inhibition of the maturation of human oncogenes

Funding Scheme: FP7-PEOPLE-2008-IRG
Period covered: 01.09.2008 – 31.08.2011
Beneficiary:
Person in charge of scientific aspects: Title:
First name: Michael
Name: Plevin
Tel: +33 4 38 78 36 98
Fax: +33 4 38 78 54 94
E-mail: michael.plevin@ibs.fr
Project website address: N/A

1. FINAL PUBLISHABLE SUMMARY REPORT

A short summary aimed at the general public

The growth, function and death of cells in multi-cellular organisms like humans need to be tightly controlled. Key to this are the mechanisms for accessing and utilizing the information stored in our genomes. One recently identified system, which regulates many different cellular processes, involves RNA, a versatile polymer made from different combinations of 4 repeating units. A large array of these regulatory RNA molecules has been identified including some which are implicated in cancers. Indeed, measuring the cellular levels of these molecules can be a useful cancer diagnostic. Cells produce each of these RNA molecules using the same biosynthetic system. My research has revealed atomic resolution insights into factors that define how these molecules are produced. If we can understand the common and unique themes we could use this information to design molecules that selectively inhibit the synthesis of oncogenic RNA regulators implicated in the development of cancers.

Scientific summary of subject

Micro-RNAs (miRNAs) are a recently identified class of small non-coding RNAs that are believed to regulate the expression of up to 30% of human genes. The contribution of miRNAs in gene regulation is gaining considerable attention, particularly given the growing links between miRNA mis-function or expression and disease. To date around 800-1000 miRNAs have been discovered or predicted in humans. The importance of their role in cellular regulation is evidenced by the genes they regulate (p53, pten, retinoblastoma, ras, etc), the processes they control (cell cycle, angiogenesis, apoptosis, etc), and the diseases to which their malfunction have been associated. A clear link exists between miRNA misfunction and cancer. Certain MiRNAs have been shown to have oncogene- or tumour suppressor-like activities. Changes in the activity or cellular levels of certain miRNAs has been implicated in different leukemias and lymphomas, and breast, lung, brain, colon and pancreatic cancers (to name a few).

Mature miRNAs are 20-25 nucleotide single-stranded molecules that function in association with the protein complex, RISC. The majority of miRNAs are genome-encoded and expressed as long 1-2 kilobase (kb) RNA primary miRNA (pri-miRNA) transcripts. The final miRNA sequence is located in the pri-miRNA and excised in a 2-step process performed by two RNase III-containing protein complexes. The first step is performed by Drosha in the nucleus and the second by Dicer in the cytoplasm. Each processing enzyme is associated with one or more accessory proteins. Almost all known miRNAs pass through this processing pathway making miRNA biogenesis an important research focal point. The basic miRNA biogenesis pathway as been mapped out and the 3D structures of several of the components have been elucidated. However, despite this work, many of the underlying mechanisms remain unclear. An atomic resolution understanding the molecular basis of miRNA biogenesis will drive future efforts to externally control the production and, therefore, level and activity of oncogenic miRNAs.

Scientific summary of research conducted and results obtained

To date my research has focused on TRBP, a multi-domain accessory protein associated with Dicer. I have used a semi-automated procedure to design and produce an array of constructs of TRBP that contain different combinations of the three double-stranded (ds) RNA binding domains (dsRBDs). I have used these to evaluate the structure and interactions of the miRNA binding region of TRBP (dsRBDs 1 and 2) using different

biochemical and biophysical techniques. The two dsRBDs that form the miRNA interaction region of TRBP fold and function independently. Analytical size exclusion chromatography demonstrates that there are no inter-domain contacts and NMR analysis reveals that each isolated domain independently adopts a stable three-dimension (3D) structure. NMR assignments of each domain have been obtained. A comparison of the NMR data of the isolated domains and a construct containing dsRBDs 1 and 2 also confirms that no there are no interactions between the two domains.

Interactions between the miRNA-binding region of TRBP and different miRNA targets have been evaluated using multiple biochemical and biophysical techniques (Size exclusion chromatography; NMR spectroscopy; isothermal titration calorimity; filter binding assays, etc). Complexes involving two ongocenic miRNAs have been characterised: miR-155 (implicated in chronic lymphoblastic leukemia, B-cell lymphomas, breast, lung, colon cancer) and miR-16-1 (chronic lymphoblastic leukemia). The data acquired support a model in which miRNAs – either precursor (pre)-miRNAs or mature miRNA duplexes – interact with four dsRBDs. Thus, single dsRBDs form 4:1 complexes with miRNAs whereas a double domain construct containing the whole miRNA interaction region forms a 2:1 complex. NMR chemical shift mapping has been used to identify the RNA binding sites and residue that participate at the interface. These data also show that identical interaction surfaces are employed irrespective of whether a single or double domain construct is analyzed.

The first two dsRBDs of TRBP show promiscuous RNA-binding properties. As well as interacting with all pre-miRNAs and mature miRNA duplexes tested, TRBP can interact with non-physiological RNAs that are not involved in the miRNA system. Biochemical and biophysical analysis shows that the same recognition surfaces are employed and similar binding affinities are observed. Moreover, NMR spectra of TRBP in the presence of physiological or non-physiological dsRNA are essentially identical. This observation strongly suggests that each dsRBD:RNA complex formed and each corresponding interface are highly similar at the molecular level. These data indicate that the interaction between TRBP and RNA is not sequence specific and that the only important constraint is a requirement for a sufficiently large region of dsRNA. Thus the miRNA interaction region of TRBP passively recognizes canonical dsRNA and not the more extensive structural features of immature and mature miRNAs (loops, bulges, overhangs, etc).

Conclusion and Perspectives

MiRNAs are being shown to play an increasingly important role in the regulation of the expression of genomic information and consequently they are implicated in many different types of cancer. Given that the majority of known miRNAs (including oncogenic miRNAs) pass through the same biosynthetic apparatus it is vital that we have an accurate, atomic resolution understanding of how this system works. My research is helping to understand the mechanisms by which these regulatory molecules are produced. By using biophysical tools like NMR spectroscopy in combination with other techniques I have been able to acquire atomic resolution information about the nature and formation of protein:miRNA complexes.

Protein complexes involved in miRNA must be capable of recognizing a vast number of small RNAs with different structures and nucleotide sequences and somehow

differentiate target from non-target substrates. How is this achieved? The data I have collected, together with that reported by other groups, suggests that simple structural features of the miRNA are recognized by functional domains present in the processing proteins (e.g. sufficient dsRNA character, 2 nucleotide, 3'-overhangs, etc) and that these features need to be encoded by the RNA sequence in order for a substrate precursor miRNA to be processed (ref. 3).

Reintegration of the Researcher

A major objective of this funding was to reintegrate a European scientist who had conducted post-doctoral research outside the E.U. This has been achieved. The Researcher has successfully integrated into the host institute which is currently supporting his application for a permanent position with the French research agency, CNRS.

2. USE AND DISSEMINATION OF FOREGROUND

Section A (public) – – DISSEMINATION MEASURES

This section should describe the dissemination measures, including any scientific publications relating to foreground and specify any applications for patents etc. Its content will be made available in the public domain thus demonstrating the added-value and positive impact of the project on the European Community.

Dissemination activities

Oral Presentations at International and National Conferences

Annual meeting of La Société Française de Biochémie et Biologie Moléculaire
Dourdan, France (November, 2010)
Title: Structural insights into RNA recognition by microRNA biogenesis proteins in plants and humans
Authors: **Michael J. Plevin**, Rodolfo M. Rasia, Matthieu Benoit, Lionel Imbert, Nicolas G. Bologna, Javier F. Palatnik, Jérôme Boisbouvier

International Conference on Structural Genomics
Toronto, Canada (May, 2011)
Title: *SeSEAM: a Systematic Mutagenesis-Driven Strategy for Site-Resolved NMR Studies of Supramolecular Assemblies*
Authors: **Michael J. Plevin**, Carlos D. Amero, M. Asunción Durá, Marjolaine Noirclerc-Savoye, Arnaud Perollier, Benoit Gallet, Thierry Vernet, Bruno Franzetti, and Jérôme Boisbouvier

Poster Presentations at International and National Conferences

Annual meeting of La Société Française de Biochémie et Biologie Moléculaire
La Grande Motte, France (September 2008)
Title: Structural Evaluation of the Molecular Interaction Networks Underlying miRNA Biogenesis
Authors: **Michael J. Plevin**, Rodolfo Rasio, Lionel Imbert, Dominique Marion & Jérôme Boisbouvier

EU-NMR Annual Meeting
Autrans, France (January, 2009)
Title: An efficient strategy for parallel screening of protein constructs by NMR.
Authors: **Michael J. Plevin**, Rodolfo Rasia, Marjolaine Noirclerc-Savoye, Benoit Gallet, Thierry Vernet, Bernhard Brutscher and Jérôme Boisbouvier.

Annual meeting of La Société Française de Biochémie et Biologie Moléculaire
Dourdan, France (November, 2010)
Title: Structural studies of human microRNA biogenesis proteins
Authors: Matthieu Benoit, Jérôme Boisbouvier, **Michael J. Plevin**

Accepted publications relevant to the funded project

1. Amero, C.D., Durá, M.A., Noirclerc-Savoye, M., Perollier, A., Gallet, B., **Plevin, M.J.**, Vernet, T., Franzetti, B., and Boisbouvier, J. (2011). *A Systematic Mutagenesis-driven Strategy for Site-Resolved NMR Studies of Supramolecular Assemblies.* **J. Biomol. NMR** 50, 229-36
2. Ayala I., Hamelin O., Amero C.D., Franzetti B., **Plevin, M.J.**, Boisbouvier J., Gans P. (2011). *An Isoleucine Labeling Strategy for the NMR Study of High Molecular Weight Proteins.* Accepted in **Chem. Comm**.
3. Gans P., Hamelin O., Sounier R., Ayala I., Durá M.A., Franzetti B., **Plevin, M.J.** and Boisbouvier J. (2010). *Stereospecific Isotopic Labeling of Methyl Groups for the NMR Studies of High Molecular Weight Proteins.* **Angew. Chem. Int. Ed. Engl**. 49, 1958-62
4. Rasia R.M., Noirclerc-Savoye M, Bologna N.G., Gallet B, **Plevin, M.J.**, Blanchard L, Palatnik J.F., Brutscher B, Vernet T, Boisbouvier J. (2009). *Parallel screening and optimization of protein constructs for structural studies.* **Prot. Sci**. 18, 434-9.

Publications in preparation or submitted relevant to the funded project

A. Benoit, M, Imbert, I, Palencia, A, Perard, J, Boisbouvier, J. and **Plevin, M.J.*** (2011). *Structural Characterization of the miRNA Recognition Region of Human TRBP.* In preparation
B. Benoit. M and **Plevin*, M.J.** (2011). *Backbone Resonance Assignments of the Micro-RNA Recognition Region of Human TRBP2.* Submitted to **Biomol. NMR Assign.**

EXPLANATION OF THE USE OF RESSOURCES

TABLE 3.1 PERSONNEL, SUBCONTRACTING AND OTHER MAJOR DIRECT COST ITEMS FOR CEA FOR THE PERIOD

PROJECT N° 231082 oncoMiRNA-biogenesis

Work Package	Item description	Amount	Explanations
	Salary of the researcher	75 000 €	Salary of the researcher, 100% funded by fellowship from l'Association pour la Recherche sur le Cancer (www.arc-cancer.net)
	Other personnel costs	23 601 €	Salary od Phd student
	Travel	96 €	Travel
	Consumable	49 861€	Chemical products : 24611 € - Small equipements and consummables for biology : 25250 €
	TOTAL COSTS[1]	148 558 €	

[1] Total direct costs have to be coherent with the directs costs claimed in Form C