Biologie intégrative du métabolisme des ARN

Rapport Hcéres

agence d'évaluation de la recherche
et de l'enseignement supérieur

Section des Unités de recherche

Evaluation report

Research unit:

Génétique moléculaire et intégration des fonctions cellulaires - Institut André Lwoff Villejuif

of University Paris 11

Mars 2009

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Le Président
de l'AERES

Jean-François Dhainaut

Section des unités
de recherche

Le Directeur

Pierre Glorieux

Mars 2009

Evaluation report

The research unit :

Name of the research unit : Génétique moléculaire et intégration des fonctions cellulaires

Requested label : UMR CNRS

N° in case of renewal : FRE 2937

Head of the research unit : M. Francois DAUTRY

University or school :

Université Paris 11

Other institutions and research organization:

CNRS

Date of the visit :

27 November 2008

Members of the visiting committee

Chairman of the commitee :

M. Laszlo TORA, CNRS, Institut de génétique et de biologie moleculaire et cellulaire, Illkirch, France

Other committee members :

M. Anders LUND, Biotechnology research and innovation center, Copenhagen, Danemark

M. Joan BURNSIDE, Delaware biotechnology institute, Newark, USA

Ms. Marie-Noëlle PRIOLEAU, CNRS, Institut Jacques Monod, Paris, France

M. Gunter MEISTER, Max-Planck biochemistry institute, Martinsried, Germany

CNU, CoNRS, CSS INSERM, représentant INRA, INRIA, IRD.....) representatives :

M. Jean-Jean OLIVIER, CoNRS representative

M. Olivier OUDAR, CNU representative

Observers AERES

M. Philippe BOUVET

University or school representative:

M. Pedro DE OLIVIERA, Université Paris 11

M. Dominique EMILIE, Université Paris 11

Research organization representative :

Ms. Martine DEFAIS, CNRS

Evaluation report

1 • Short presentation of the research unit

- Number of lab members 27: including
  - 10 researchers with teaching duties and full time researchers
  - 5 PhD students, all with a fellowship
  - 5 engineers
  - 6 technicians
  - 1 administrative assistants
- Number of HDR: 7
- Number of PhD students who have obtained their PhD: 8
- Average length of a PhD during the past 4 years; 3,5 years
- Number of PEDR : 1
- Number of "publishing" lab members: 9 out of 10

2 • Preparation and execution of the visit

The committee visited the laboratory on the 27th of November 2008. The visit was well prepared, with a thorough and detailed document provided in advance. On site, the visit was properly organized, with presentations and discussions with group leaders, technical and administrative staff, students and postdocs. The director gave his presentations in front of the committee and in the presence of the other members of the Unit. The committee had time to discuss various issues and the execution of the visit was thus good. No committee visit was organized to visit the different separate buildings of the Unit.

3 • Overall appreciation of the activity of the research unit, of its links with local, national and international partners

Overall, the research carried out at the unit can be qualified as good. The committee noticed a substantial heterogeneity in the performances in the various groups, yet the general feeling was rather positive. While some groups are clearly at the international standard, others experience some difficulties to reach a level of visibility, which may be expected for a research unit in France. This is also visible when one considers the level of funding that various groups can attract into the institute as well as the recent track records of the PIs.

The difficulties of some of the teams can be partially explained by the important turn over and reorganization that this unit has experienced lately. Out of the four groups that were qualified as groups during the past four years only two will continue to exist in the new plan, and even one of the two has changed its scientific orientation. One previously existing group (Differentiation of stem cells) will leave and one other previous group (Mitotic molecular motors) has already incorporated in a novel group (Compartmentation and intracellular traffic of mRNP) that grew out from the group called "Post-transcriptional regulation". The group previously called "Post-transcriptional regulation" has been subdivided in three novel groups: "Regulation of small RNAs", "Compartmentalization and traffic of mRNPs", and "Transcription and cellular interactions". The below team-by-team assessment will be done according to these four new groups since the presentations were done by these four group leaders.

The challenge for this institute is to take advantage of this novel changing situation to impose a new momentum and create a dynamic of success. The committee thinks that human resources are there to make this possible.

It is also worth mentioning that in the past four years the head of the unit was deeply involved in the administration of research at the University and this at an exceptionally high degree. His duty is now finished and thus the director should have more time to concentrate on the scientific and administrative duties of the unit.

Regarding the educational aspect, the committee was glad to meet the enthusiastic doctoral students. It appears that the tutorship is well done and that PhD studies at the unit are well organized and supervised. Students are encouraged to be actively involved in the life of the institute, through a variety of activities.

4 • Specific appreciation team by team and/or project by project

Group 1: Regulations by small RNAs

The lab works on regulation mechanisms of gene expression by small RNAs. The group consists of the head, one technician and two students who are working on their PhD thesis. At least three individual projects are currently carried out in the lab.

1. Kinetic analysis of RISC cleavage

Kinetics of RISC cleavage have been analyzed in living cells using a tetracycline inducible promoter and the beta-globin mRNA. RISC cleavage seems to be rather slow compared to other cellular processes.

2. Nuclear activities of small RNAs

Nuclear RNAi is investigated in this project. They find that nuclear RNAi is as active as cytoplasmic RNAi, which as been observed by others as well.

3. Post-transcriptional and transcriptional activities of small RNAs

By using a bi-directional promoter, transcription and silencing effects will be measured simultaneously. Transcriptional effects caused by siRNAs have already been confirmed and the detailed mechanisms of small RNA-guided transcriptional silencing will be investigated.

— Strong points :

The lab has chosen the emerging field of “small RNAs” as research topic. This is very ambitious since many important discoveries are still to be made in this field. At least some of the presented projects are high-risk projects. Sometimes high-risk projects lead to a high-gain of knowledge since such projects may open-up new fields. Indeed, the existence of small RNAs in the nucleus as well as small RNA-guided transcriptional silencing phenomena are highly controversial in the field. Clear and straightforward experiments that explain such phenomena would certainly be a break through. However, the outcome of these experiments might be that there is no small RNA function in the nucleus. It is also positive that two graduate students are hosted in the lab.

— Weak points :

A group with two students and one technician is simply too small to be competitive in the RNAi field. It is not very likely that a high impact publication will be produced in the next years. It is very important that the group will be restructured and more experienced post-docs focus on individual projects. Moreover, high-risk and low-risk projects are not balanced in the lab. At least one low-risk project should be established that ensures constant and high-quality publications. Another weak point is, that the publication record of the lab during the last four years was rather weak. This needs to be improved. Finally, there is no home page or other Internet appearance of the lab (at least none that is easily found). This would be extremely important for interested students or post-docs.

— Recommendations :

In summary, it would be better to focus on one (or two) individual project(s) instead of working on many in parallel. The group size is simply too small to be competitive in the small RNA field. Therefore, more students

and post-docs should be hired to generate a critical mass of people. Teaming up with other labs either at the institute or at other institutes would certainly be beneficial for the lab as well.

Nom de l'équipe : Regulations by small RNAs

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
B	B	B	B	B

Group 2: Replication-transcription coupling and Ultrastructure of the cell

The team has developed two unrelated activities: one dedicated to the analysis of the ultrastructure of the cell by electron microscopy and one on DNA replication in *Physarum polycephalum*. The first activity mostly relies on collaborations with groups working in several fields and has led to an impressive list of publication in highly ranked journals (Lab head, one technician and two engineers). The team has been working on DNA replication in *Physarum polycephalum* for many years. The laboratory although small has very ambitious projects, one based on the development of large-scale analyses aimed at studying the connection between replication origins and promoter elements (one researcher) and one dedicated to the understanding of the structure and the role of transient post-replicative joint DNA molecules (one researcher).

— Strong points :

The team develops new tools for electron microscopy. The expertise of the team in bidimensional gel electrophoresis technique coupled to the natural synchrony of the cell cycle of plasmodium (a giant multinucleated cell) allowed them to evidence joint DNA molecules transiently formed between sister chromatids on newly replicated DNA. These junctions have been identified in a variety of organisms showing that formation of replication-associated joint DNA molecules is a general event in eukaryotic genomes. No doubt that the synchrony of the plasmodium is the best system for determining the role and the precise structure of these molecules. They further studied the structure of joint DNA molecules and demonstrated that these molecules are as abundant as replication intermediates at various loci. They showed that discontinuities are located at the branch point of these molecules and are enhanced by the addition of RNase, thus showing that joint DNA molecules contain ribonucleotides in their branch point. Their results suggest a new model in which ribonucleotides misincorporated during DNA replication are recognized by a resolvase or an endonuclease, leading to their excision. This work is not published yet but the manuscript in preparation should be published in a highly ranked journal. Their hypotheses will be tested by depleting recombination machineries with siRNA. They already have proven that siRNA is very efficient in this system.

— Weak points :

The first project depends on a good annotation of the *Physarum* genome. For this purpose, the laboratory has played an important role in initiating a project of sequencing by the NIHGR. Unfortunately, technical problems have considerably slowed down this project restricting genome-wide studies. Moreover, the group is too small to efficiently develop such an ambitious project (only one researcher). Despite the high quality of the work made in this group, the team has difficulties in obtaining funding and in attracting students or post-docs.

— Recommendations :

The committee strongly encourages the group to have a more active policy to write grants and increase the visibility of the studies carried out in the group to attract students and post-docs.

Nom de l'équipe : Replication-transcription coupling and Ultrastructure of the cell

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	B	B	A

Group 3: Compartmentation and Intracellular Traffic of mRNP

The group is headed by a senior scientist with a CNRS CR1 position. It includes 3 permanent staff scientists (1 DR2 CNRS, 2 CR1 CNRS) one technician, one PhD student and a post-doc with ANR financial support. The group studies translational regulation with special emphasis on the formation, composition and dynamics of stress granules and GW bodies. This is an internationally competitive research area of interest to both basic and translational sciences.

This is a new research group established in January 2008. The primary investigator was previously associated with another laboratory within the unit, but appears here to have conducted an independent research program. This is reflected in several scientific papers with the group leader figuring as senior corresponding author. Hence, over the last years the group leader has established herself as an international capacity in the research field. The group has produced 9 scientific papers in the period 2004-2008, 4 of which with the group leader as senior corresponding author. The papers are of high standard and appear in internationally recognized and peer-reviewed journals, such as *Molecular Biology of the Cell* and *Journal of Cell Science*. The group has established several scientific collaborations both within the institute and internationally.

To date the scientific research tools and experimental readouts have to a large extend been centred around microscopy and image analysis, making use of overexpression or RNAi-mediated knockdown to perturb the system in study. In the proposed project the experimental approaches are expanded to include both detailed biochemical analyses, genetic analyses including extensive transcriptome profiling of mRNAs and microRNAs and a series of functional studies.

— Strong points :

The proposed research project is both ambitious and very challenging, as several new techniques will have to be established within the laboratory or through collaborations. The vast broadening of the experimental approaches will generate a large quantity of data and likely provide several new research options. Hence it will be of outmost importance to maintain a clear focus with continuous prioritization of the subprojects.

However, based on the previous track record, the committee finds it feasible that the group will be able to conduct the proposed experiments within a 4-year period given the manpower available and collaborations established.

— Weak points :

This group should accommodate more than one PhD student.

— Recommendations :

In summary, the committee evaluates this to be a dynamic and vigorous research group with a strong research program in an important research field and with good chances of future success.

Nom de l'équipe : Compartmentation and Intracellular Traffic of mRNP

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	B	A

Group 4: Transcriptome and cellular interactions

The team has been studying the molecular aspects of genetic resistance to disease in chickens using a transcriptome profiling approach. Chickens represent an excellent model system for these studies as the genetics are well defined with respect to the contribution of the MHC to resistance. In addition chickens are available in very large numbers since they represent an economically important protein source. Finally, diseases affecting chickens not only have financial impacts for the industry, but birds can also serve as reservoirs for human pathogens.

— Strong points :

This team embraced challenging technology to develop tools and resources for chicken immunology and has applied this to gene expression profiling during the course of infection with an avian pathogen. This has produced one manuscript in Journal of Virology (Impact factor of JV=5.3; this paper has two citations).

The lab has established good collaborative projects nationally and internationally.

The group leader has a significant teaching responsibility and appears to be an energetic and motivated instructor. There are two graduates students in the team, and both were enthusiastic and knowledgeable about their research.

The noncoding RNA work is an exciting new area for the team. This work is described in a new Immunogenetics paper, which also reports cDNA library construction, sequencing and analysis, microarray and Northern expression analysis and extraction of candidate ncRNAs, validation. This study represents a very large volume of work.

— Weak points :

The continued use of in house designed/fabricated cDNA microarrays was not considered state-of-the-art, as technology and available resources have advanced since the project was initiated. Continued use of these arrays because the team finds the size of the datasets more manageable, or because the content was immune system targeted, was not considered to be a good justification or the best approach for transcriptome analysis.

Review panel members shared the concern that the transcriptome studies were not hypothesis driven, and the next steps to be taken as follow up to existing observations were not clear in either the presentation or in the publications.

The identification of noncoding RNAs from the lab's small EST collection (~11,000 clones) rather than the entire collection of ESTs in GenBank (>500,000) was considered a limited approach. The current bioinformatics pipeline contains a manual step, which could be automated to accomplish a more comprehensive evaluation, and could also provide tissue expression profiles. Additionally, the current analysis has produce a number of potentially novel and important observations but no experiments for follow-up studies were presented.

— Recommendations :

The group leader should build a clear focus that is based upon current work and establish testable hypotheses. In addition, the work using arrays should embrace more current technology. An increase in the number of publications with the group leader as senior author would increase the visibility of the program.

Nom de l'équipe : Transcriptome and cellular interactions

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
B	B	B	B	B

5 • Appreciation of resources and of the life of the research unit

This is a small unit with four groups, dispersed in several buildings of the Villejuif campus.

There has been a gradual erosion in institutional fundings, which should be closely monitored by the CNRS since a continuation of this trend will significantly reduce competivity of the unit as a whole. 80 % of the unit's budget comes from outside grants. Funding for renovation of certain parts of the unit has to be integrated into a campus-wide policy.

Discussion with PIs, tenured staff, technical and administrative staff, post-doctoral and doctoral students indicated a good degree of cohesion and interaction within the unit and a general contentment in the way in which the unit functions.

6 • Recommendations and advice

— Strong points :

There is a good coherence amongst projects suggested by the four groups. In the plan presented for the next four years the groups tried to coordinate their future research fields to connect better to each other. However, the committee felt that sometimes the connections were somewhat artificial. Most of the groups have an exstensive network of collaborations. The unit has a very good electron microscopy service with a very long standing expirience. In general the scientists and the staff is happy to work in the unit.

— Weak points :

The institute has very limited international visiblility. In the groups very few Ph.D. students and post-doctoral fellows are hired. Some of the labs are in bad shape. The groups are dispersed. In the same buildings another unit is working on similar projects using often similar methods with whom the evaluated unit (FRE 2937) has almost no contacts. Out the the four new groups presented three spun off from one previous group. There is very little mobility. Some of the presented projects lack sufficient focus.

— Recommendations :

The committee thinks that the unit should increase its visibility and recruit more Ph.D. students and post-docs. Most of the PIs of the unit should focus better their projects and also write more grants to attract more outside fundings (containing salaries for students and post-docs).

The institute is going now through a critical phase in its re-organisation and the committee thinks that the support should be maintained. The critical phase for the institute will be in the coming 4 years and we believe that the management should be given the appropriate tools to re-enforce the actual qualities and implement novel research avenues.

Note de l'unité	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
B	B	B	B	B

Génétique Moléculaire et Intégration des Fonctions Cellulaires
FRE 2937

Comments on the report of the visiting committee of FRE 2937 « Génétique moléculaire et intégration des fonctions cellulaires"

We appreciate that the experts were chosen in order to cover the different activities of the research unit; however, we strongly regret that, in some cases, this search has led to a conflict with the neutrality which is required for an evaluation. This has generated two types of problem in the report.

i) A lack of objectivity in the analysis of the activity of group 1

A growing body of literature published in high profile journals implicates nuclear RNAi in several aspects of gene regulation in mammals. Accordingly, it is first written that some of the results of this group have "been observed by others as well". However, later on it is stated that "the outcome of these experiments might be that there is no small RNA function in the nucleus", which reflects a personal opinion rather than an evaluation of the current state of this field.

ii) A conflict of interest in the analysis of the activity of group 4

This group aims to generate unbiased transcriptome analysis of the response to infection of different strains of chicken. These will be used for further studies within several European projects including one on avian flue which is not even mentioned. The use of "in house" micro arrays was not considered state-of-the art. However, the incriminated micro arrays use oligonucleotides covering the whole chicken coding transcriptome. They are continuously developed and produced as part of a European project which is coordinated through a network of excellence (EADGENE). Thus it appears that this statement reflects a conflict between two strategies, a European one, which is based on a public consortium and an American one, which relies on commercial arrays based on an incomplete genomic sequence. For the non-coding transcriptome, although the initial study was performed on an immune centred data set, it has been clearly indicated that the main goal was to extend this study to the whole genome. Finally, isolating one publication in J. Virol (top ranking journal in virology) to state that it has only two citations so far (six currently) is ridiculously restrictive and the major visibility of the group leader easily emerges in any type of more extensive analysis. More generally, the track record of group 4 can be appreciated through its active role in European projects and this seems to be dismissed by the report.

François Dautry, Director of FRE 2937

Institut André Lwoff
7 rue Guy Môquet - 94801 Villejuif Cedex – France
Tel : 33 (0)1 49 58 36 99
Fax : 33 (0)1 49 58 33 81