# Réparation du génome mitochondrial normal et pathologique

Rapport Hcéres

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

Section des Unités de recherche

# AERES report on the research unit

## Repair in Normal and Pathological Mitochondrial Genome

## From the

## Blaise Pascal University

March 2011

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## From the

## Blaise Pascal University

Le Président de l'AERES

Didier Houssin

Section des unités de recherche

*Le Directeur*

Pierre Glorieux

March 2011

# Research Unit

Name of the research unit: Repair in Normal and Pathological Mitochondrial Genome

Requested label: EA

N° in the case of renewal

Name of the director: Mr. Patrick VERNET

# Members of the review committee

## Committee chairman

Mr. Pierre COUBLE, Center for Molecular and Cellular Genetics, Lyon. France

## Other committee members

Mr Zoran CULIG, Innsbruck Medical University, Austria

Mr Antoine GUICHET, Jacques Monod Institute, Paris, France

Mr Thierry GRANGE, Jacques Monod Institute, Paris, France

Mr Ivan TARASSOV, Molecular Genetics, Genomics and Microbiology, Strasbourg

Ms. Marie-Agnès SARI, Paris Descartes University, Paris. France

# Observers

## AERES scientific advisor

Mr. Jean-Antoine LEPESANT

## University representative

Ms. Pascale DUCHE, Université Blaise Pascal - Clermont-Ferrand II

# Report

## 1 • Introduction

- Date and execution of the visit

The visit took place in Clermont Ferrand, on the Campus Les Cézeaux, on Wednesday 2nd of March 2011. It started with the audition of the Director and was followed by a separate meeting with all personnels of the group. After a discussion with the President of the Scientific Council of the University Blaise Pascal, the Committee had a final closed-door meeting to draw the main conclusions.

- History and geographical localization of the research unit, and brief presentation of its field and scientific activities

The proposed project is in the continuity of that of a team presently hosted in the GReD unit (CNRS 6247/INSERM U931). It emerged after the retirement of the professor who assumed for the past years the team leadership and the recruitment of a new Professor of the University Blaise Pascal. The selected new group leader was also working in the GReD but on an unrelated project.

The recruited leader has worked since 2009 at building up a novel research team with the status of University-team (Equipe d'accueil) that would be located in the Plant Biology building on the Campus Les Cézeaux of the University Blaise Pascal –Clermont II .

The main project concerns the unraveling of the mechanisms of DNA repair in the mitochondrion, using essentially cell lines from both Drosophila and healthy or tumorous human prostate.

- Management team

The new team leader was a specialist of the effects of free radicals on sperm maturation during the passage through epydidymis. His investment in the new project implied that he changed his main scientific interest toward the biology of DNA repair.

- Staff members

| | Past | Future |
|---|---|---|
| N1: Number of researchers with teaching duties (Form 2.1 of the application file) | 6 | 5 |
| Number of full time researchers from research organizations (Form of the application file) | 0 | 0 |
| N3: Number of other researchers including postdoctoral fellows (Form 2.2 and 2.4 of the application file) | 0 | 0 |
| N4: Number of engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file) | 0,45 | 0,45 |
| N5: Number engineers, technicians and administrative staff without a tenured position (Form 2.6 of the application file) | 0,5 | |
| N6: Number of Ph.D. students (Form 2.7 of the application file) | 1 | |
| N7: Number of staff members with a HDR or a similar grade | 2 | 1 |

## 2 • Overall appreciation on the research unit

- Summary

The proposed project for the creation of a University team (Equipe d'accueil) is based on the acquired know-how of an existing group presently at the GReD, with extended expertise in Drosophila genetics and mitochondria-related methods.

The project aims at better comprehend the molecular mechanisms of mitochondrial (mt) DNA repair, especially the correction of double strand breaks, in normal and pathological states. It exploits two model systems : Drosophila cultured cells and human prostate cells lines, either healthy or tumorous. The team is composed of a professor and four assistant-professors (MCF), and benefits from a part-time technical assistance.

- Strengths and opportunities

The current knowledge of the pathways mobilized in mitochondrial DNA repair is very poor. The default in repairing mitochondrial DNA lesions is often associated with the emergence of pathologies, but the understanding of the concerned pathways remains to be worked out. Therefore, it is sound that more groups invest in this field, eventhough a competitive context is driven by a few outstandings groups in Europe and the rest of the world. The past experience of the team in Drosophila mitochondria functions is also an asset.

- Weaknesses and threats

The scientific project rests upon the value of the Drosophila model system (mostly approached via the exploitation of S2 cultured cells) to better comprehend the mechanisms at work in human prostate cancer cells lines. Though many actors of DNA repair are conserved through evolution, the phylogenetic distance between fly and man let suspect that species-specific pathways may be recruited in mtDNA repair, which questioned both the pertinence of working in parallel with an invertebrate and a mammalian system, and therefore the feasability of the research program.

The study of mt DNA repair in prostate cancer cell lines and its implication in carcinogenesis would have deserved to be more accurately substantiated and still remains to be matured. For instance, a more detailed presentation of the current knowledge and preliminary data in the proposal would have been helpful to appreciate the scientific potential.

Except for the present team leader who joined recently and performed previously very well in another research field, the accomplishments of other staff members of the team in the production of publications, the training of Thesis or Master students, the welcoming of post doctoral fellows and the raising of funds for the project have been low up to now.

The project is significantly based on collaborations which still await specific financial support and whose efficiency remains to be proven. The Principal Investigator may wish to approach the French ARTP (National Organization for Prostate Cancer Research), which distributes grants and organizes scientific meetings.

- Recommendations

The Committee felt that the project would be more feasible if only one biological system were explored. Indeed, it is not certain that the team has the capacity to carry a deep exploration of the two distant models. It is recommended to concentrate on human cell lines with DNA micro-array and to compare the performance in mtDNA repair pathways in normal and tumour cell lines. Alternatively, the experience of the group on the Drosophila model could be exploited if the genetic potential of the fly and the generation of novel mutants affected in mtDNA repair are considered.

The team should deploy considerable efforts to make the project feasible. This concerns:

- the raising of specific research funds, in particular in the context of the expected drastic decrease in the institutional credits available.
- the hiring of Master and Thesis students, which implies that staff members, other than the team leader, work at being habilitated to mentor PhD candidates.
- the establishment of official and funded collaborations, in particular with the CEA group in Grenoble.
- A better integration within the international community working on mtDNA repair, which requires that the team members participate now to European and International scientific meetings in the field.

- Production results

| | |
|---|---|
| A1: Number of permanent researchers with teaching duties (recorded in N1) who are active in research | 4 |
| A2: Number of permanent researchers without teaching duties (recorded in N2) who are active in research | 0 |
| A3: Ratio of members who are active in research among staff members [(A1 + A2)/(N1 + N2)] | 0,7 |
| A4: Number of HDR granted during the past 4 years | 1 |
| A5: Number of PhD granted during the past 4 years | 0 |

## 3 • Specific comments

- Appreciation on the results

The results of the 5 last years described in the report concern essentially the work of the "Mitochondrial Genome" group still currently in the GReD unit for the remainder of the four-year contract. The past projects of the team aimed at understanding (1) the mechanisms of the decay of activity of the respiratory complex I during aging in the Drosophila model and (2) the nature of the proteins involved in the processes of mitochondrial DNA double strand break (DSB) repair in the Drosophila S2 cell line and, more recently, in human prostate tumor cell lines.

Project (1) led to hypothesize that age-dependent enzymatic decay is caused by a general decrease of mtDNA transcription, which, in turn, could be the consequence of the lowered level of mitochondria-imported transcription factors. Two papers were published in a generalist journal (Biochimie) and a specialized one (In Vitro Cell Dev Biol Anim).

Project (2) used an original system of bleomycin-induced mtDNA DSB followed by siRNA driven depletion of candidates for the role of repair proteins. This was described in a publication in a specialized journal (Mutat Res). The system was also applied to analyze in silico predicted candidates (not published yet). The same bleomycin-induced DSBs were tested in human prostate cell lines, with the objective to study the functioning of the mitochondrial repair system upon carcinogenesis (not published yet).

Overall, results were presented at the editions of the Congrès du Groupe Français de Bioénergétique (GFB) in 2007 (one oral communication, two posters) and in 2009 (two posters).

Partnerships essentially concern a not yet funded cooperation with a CEA group in Grenoble that masters an original technology of DNA modified chips, and the participation, together with the Laboratoire de Physique Corpusculaire, in the setting up of a local gamma-irradiation platform PAVIRMA, which is expected to be functional early 2011, but whose final installation is planned in three years.

On the other hand, the new head of the group, formally invested in the study of the effects of free radicals on spermatozoids in the mouse epydidymis published 9 papers, including a high impact one (JCI).

- Appreciation on the impact, the attractiveness of the research unit and of the quality of its links with international, national and local partners

Neither prizes nor particular distinctions were awarded to the team members. One oral presentation directly related to the present research project was given at the national GFB congress in 2007.

Over the same period, the proposed new team leader participated as a speaker in two international and two national scientific symposia, still as a member of his former GReD group working on sperm maturation.

The team, composed of teaching staff of the University Blaise Pascal, was joined by another scientist of the GReD to take over the direction of the group. Otherwise, no specific recruitments were carried out, while some decrease in personnel occurred (in the scientific, as well as in the technical staff). Only three M2 students were recruited in the past 5 years and only one thesis student has joined the team in 2010. Among all the teaching staffs, only the team leader has a HDR. Neither postdoctoral students nor invited scientists were mentioned.

The team did not obtain any significant project-specific funding (with the only exception of 15 k€ from the Ligue Contre le Cancer), and functioned essentially on University and CNRS/INSERM recurrent budgets.

Its local involvement in the emergence of the above-mentioned PAVIRMA platform is significant.

- Appreciation on the management and life of the research unit

The organization of the laboratory unit is essentially that inherited from the existing group still at GReD, except for the change in the group leader. Although he had not a specific expertise in the research project when he joined in 2009 as a University professor, the present leader played a positive role in scientific animation and contributed strongly to the structuring of the future project.

The connection of the members of the teaching staff with the University Blaise Pascal is very good, and several of them are committed to collective responsibilities in the organization of master courses and in the Department of Biology. The heavy investment of all the teaching staff in pedagogy is however a difficulty that may impair the progression of the project.

- Appreciation on the scientific strategy and the project

The project is based on the past experience of the existing mitochondrial group with an expertise in Drosophila genetics and mitochondria-related methods. The new project is re-axed on studies of genetic and molecular mechanisms of DNA repair in mitochondria, in normal and pathological states of the cell. It rests upon the use of Drosophila (mostly approached via established cell lines) as a model system to better decipher the recruitment of mtDNA repair pathways in mitochondria of human prostate cells lines, healthy or tumorous.

The rationale for using such phylogenetically distant systems is not clear, owing to the probable existence of species-specific DNA repair pathways that could contradict the choice of Drosophila to help unravel mechanisms that prevail in human.

In addition, how the project will contribute to enlighten the role of mtDNA repair mechanisms in carcinogenesis is not mature enough. To identify systems related to modified bases repair, it is proposed to launch a collaboration with a CEA (Grenoble) laboratory and use their chips to analyse mitochondrial proteins; while this may lead to delineate pertinent related enzymatic activities, the identification of the cognate proteins involved in DNA repair may be difficult, especially for unknown actors for which no mutants are available.

The resources in terms of permanent staff appear sufficient to pursue the ambitious scientific aims. On the other hand, the quasi-absence of Thesis students or postdoctoral fellows is a serious handicap that should be carefully looked at. The resources in terms of general functioning and equipment will depend on the availability of local platforms, on the success of newly launched collaborations and on the ongoing grant applications mentioned in the project. It is clear that in order to develop a competitive project of such an ambition, the team must make a strong effort to join the international community working in the field.

| Intitulé UR / équipe | C1 | C2 | C3 | C4 | Note globale |
|---|---|---|---|---|---|
| RÉPARATION DU GÉNOME MITOCHONDRIAL NORMAL ET PATHOLOGIQUE | B | B | B | B | B |

**C1** Qualité scientifique et production

**C2** Rayonnement et attractivité, intégration dans l'environnement

**C3** Gouvernance et vie du laboratoire

**C4** Stratégie et projet scientifique

## Statistiques de notes globales par domaines scientifiques

*(État au 06/05/2011)*

### Sciences du Vivant et Environnement

| Note globale | SVE1_LS1_LS2 | SVE1_LS3 | SVE1_LS4 | SVE1_LS5 | SVE1_LS6 | SVE1_LS7 | SVE2 _LS3 * | SVE2_LS8 * | SVE2_LS9 * | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| A+ | 7 | 3 | 1 | 4 | 7 | 6 | | 2 | | 30 |
| A | 27 | 1 | 13 | 20 | 21 | 26 | 2 | 12 | 23 | 145 |
| B | 6 | 1 | 6 | 2 | 8 | 23 | 3 | 3 | 6 | 58 |
| C | 1 | | | | | 4 | | | | 5 |
| Non noté | 1 | | | | | | | | | 1 |
| **Total** | **42** | **5** | **20** | **26** | **36** | **59** | **5** | **17** | **29** | **239** |
| A+ | 16,7% | 60,0% | 5,0% | 15,4% | 19,4% | 10,2% | | 11,8% | | 12,6% |
| A | 64,3% | 20,0% | 65,0% | 76,9% | 58,3% | 44,1% | 40,0% | 70,6% | 79,3% | 60,7% |
| B | 14,3% | 20,0% | 30,0% | 7,7% | 22,2% | 39,0% | 60,0% | 17,6% | 20,7% | 24,3% |
| C | 2,4% | | | | | 6,8% | | | | 2,1% |
| Non noté | 2,4% | | | | | | | | | 0,4% |
| Total | 100,0% | 100,0% | 100,0% | 100,0% | 100,0% | 100,0% | 100,0% | 100,0% | 100,0% | 100,0% |

*** les résultats SVE2 ne sont pas définitifs au 06/05/2011.**

## Intitulés des domaines scientifiques

### Sciences du Vivant et Environnement

**• SVE1 Biologie, santé**
- **SVE1_LS1 Biologie moléculaire, Biologie structurale, Biochimie**
- **SVE1_LS2 Génétique, Génomique, Bioinformatique, Biologie des systèmes**
- **SVE1_LS3 Biologie cellulaire, Biologie du développement animal**
- **SVE1_LS4 Physiologie, Physiopathologie, Endocrinologie**
- **SVE1_LS5 Neurosciences**
- **SVE1_LS6 Immunologie, Infectiologie**
- **SVE1_LS7 Recherche clinique, Santé publique**

**• SVE2 Ecologie, environnement**
- **SVE2_LS8 Evolution, Ecologie, Biologie de l'environnement**
- **SVE2_LS9 Sciences et technologies du vivant, Biotechnologie**
- **SVE2_LS3 Biologie cellulaire, Biologie du développement végétal**

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Monsieur Pierre GLORIEUX
Directeur de la section des Unités de Recherche
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75002 Paris

Monsieur le Directeur,

Nous nous associons aux remerciements émis par la direction du laboratoire **"Réparation du Génome Mitochondrial Normal et Pathologique"** (demande de création d'EA) aux membres du comité d'évaluation pour la qualité de leur travail. Vous trouverez ci-jointe la réponse de l'équipe de direction du laboratoire.

Nous partageons l'avis du comité selon lequel l'équipe possède de véritables atouts pour son développement, en particulier sa connaissance de la fonction mitochondriale en utilisant comme modèle la drosophile.

Comme le souligne le comité, le recentrage thématique qu'opérera l'unité sera certainement le gage du développement du potentiel de recherche de l'équipe.

L'université et l'unité sauront tirer profit des recommandations proposées par le comité afin de déterminer conjointement la solution optimale de faisabilité du projet.

Nous vous prions d'agréer, Monsieur le Directeur, l'expression de nos salutations distinguées.

Clermont-Ferrand, le 14 avril 2011

La Présidente,

Nadine LAVIGNOTTE.

# Project
# "Repair in Normal and Pathological Mitochondrial Genome"

# Supplemental data brought by the Unit director to the AERES report

The team leader and all the members of the group wish to thank the visiting committee for the quality of the evaluation process during the one-day meeting but also for the serious and deep analysis of our project. Overall comments and conclusions are appropriate and will be taken into account for the next 5-year period. However, we will take the opportunity to bring some supplemental comments in order to precise and complete some points from the committee report.

A. Introduction

History and geographical localization of the research unit

We would like to bring some precisions regarding the team leader arrival and the status of the new team. The leader started in the group in January 2009 as a co-director beside the previous team leader. At the end of 2009, beginning 2010, both leaders and Blaise Pascal University representatives started to work together on a new status for the group. In October 2010, the new team leader took over the full direction of the group after the retirement of the previous director.

B. Overall appreciation on the research unit

a. General remarks

The team has undertaken since 2008 a profound scientific reorientation going onto mitochondrial DNA repair. Previous works of the group were focused on ageing effects on mitochondria DNA and biochemical properties using fruit fly as a model. Both ageing and repair studies have been performed in parallel in the current 4-year contract since maintenance of mitochondrial DNA (mtDNA) is known to be modified during ageing with an increase of mtDNA damages. However, ageing studies have been progressively abandoned to clearly focus since one year on the mtDNA repair, following recommendations from the CNRS committee.

The committee underlines the poor knowledge of the scientific community on mtDNA repair mechanisms and the importance to decipher pathways involved in mtDNA maintenance. In France, this aspect is only studied in a very limiter number of groups.

Based on a strong expertise on the drosophila model, the group initiated studies on DNA repair on S2 drosophila derived cells. This initial approach was justified by the easy handling of this cell lines and the opportunity to transpose data generated on a homologous cell model to drosophila. However, aware of the limitations of such an approach and to get the full genetic potential of drosophila, we already started to work on 3 drosophila mutants (Bloomington stock centre) deficient in essential base excision repair proteins (OGG1, Thd1 and XRRC1).

Our project is based on a first approach on drosophila model to get a grip on mtDNA repair mechanisms and then to transpose acquired data on human prostate cancer cell lines. We acknowledge the phylogenetic distance between fly and man pointed out by the committee, but most of the current data acquired on nuclear DNA repair mechanisms and molecular actors found in lower Eukaryotic cells (Yeast) or Invertebrates have been then confirmed in Human. Such an approach appears pertinent even though some DNA repair candidates or mechanisms could be species specific.



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Part of the project will lead to the screening of RNAi library to target specific genes involved in mtDNA double strand breaks repair; this part will be based on the use on a restriction enzyme targeted to S2 cells mitochondria currently generated in our group. Candidates isolated through this screen will be *in vivo* tested in mutant drosophila engineered in the local Fly Facility platform.

All this approach could not be easily and directly performed in human tumour cell lines. We acknowledge the need to present more precisely the project on the prostate model. However, current available data are mainly focused on the description of mtDNA changes during tumorigenesis (in various tumour cell lines) and on consequences for cell biology. Only few data start to suggest a potential role of mitochondria DNA maintenance mechanisms in this process. This is the purpose of our project to be part of the deciphering of this new approach through (1) the collaboration initiated with the CEA group in Grenoble and (2) the use of the PAVIRMA platform. The scientific interest of this project has been recognised and funded by the Ligue Contre le Cancer (15 k€) in the past 2 years.

b. Specific points

As explained above, the team encountered a deep reorientation of its program during the last review period. This reorientation was followed by a decrease in terms of publications. However, it also leads to a publication showing for the first time the importance of double strand breaks DNA repair in *Drosophila melanogaster*. This program is now fully functional and will be efficiently valorised.

A part of our project is based on collaboration, mainly with a CEA group in Grenoble. This collaboration has been initiated one year ago. It has already been very fruitful for our group since results have been obtained on base excision repair activities in mitochondria and will be presented as a poster (Meetochondrie meeting, May 2011) and as a talk (CLARA workshop may 2011). Part of this collaboration was funded by a mobility grant (October 2010-April 2011) from the CLARA.

Regarding the recommendations from the committee:

We acknowledge the need to work on a unique biological model to gain in efficiency. The drosophila project is currently more advanced than human studies and constitutes the core centre of a Master 2 and thesis student projects. We will mainly aim to the valorisation of this project in terms of generation of biological tools but also scientific publications. This work will be completed in the next 2 years, up to 2013. In the mean time, the human model will take over and will gain in efficiency with the PAVIRMA platform.

C. Specific comments

a. Appreciation of the results

The committee correctly summarized the overall production of the group in the past 4-year contract. We would like to underline the achievement of the PAVIRMA platform where the group is a joint holder (with the Laboratoire de Physique Corpusculaire, IN2P3) since the start of the project. This project brings together the Auvergne Region, the FEDER, the CNRS and Blaise Pascal University for a total budget of nearly 730 k€. 1st phase (γ irradiation facility) will be completed in July 2011 and the 2nd phase (neutron shotgun) will be achieved during the 2nd 2012 semester. The interest for such a platform has been confirmed by proposal letters from industrials (Biogemma, Biopôle…) and academics (INRA, Centre Jean Perrin…).



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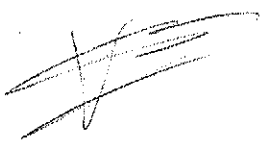
b. Appreciation on the impact, the attractiveness of the research unit and of its links with international, national and local partners

The local and national recognition of the team in the field of mitochondria biology was attested by the organisation in 2009 of the national GFB congress welcoming 80 scientists from all other France for a 3-day meeting on Bioenergetics.

c. Appreciation on the scientific strategy and the project

The committee has pointed out the quasi-absence of thesis student. This issue has been solved this year with the hiring of a PhD student funded by the government. The project started on November 2010 for a 3-year period. In the mean time, a Master 2 student is currently involved in the project of the group for the university year, and a proposal for a Master 2 project for the 2011-2012 period has been proposed. Overall, three Master 2 students have been trained in the group in the last 5 years which constitutes the mean value for Biology laboratories in Clermont-Ferrand context. However, we are aware of the lack of postdoctoral fellows in our group, this point will be prioritised mainly on the collaborative project performed with the CEA group in Grenoble. Moreover, the presence of an Emeritus Professor, fully dedicated to research within the group, since October 2010, is extremely positive.

Aubière, April 14th, 2011



Patrick Vernet, team leader

RGM

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