PASTEUR PARIS UNIVERSITE (PPU) INTERNATIONAL DOCTORAL PROGRAM 2020

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PROJECT

- **FILE #06**
- **ACRONYM:** VDJ-Rep
- **TITLE:** DNA damage/repair coupling as a safeguard against genetic instability: V(D)J recombination as a paradigm

LABORATORY

- **SURNAME, FIRST NAME:** Villartay Jean-Pierre
- **IP DEPARTEMENT:** Cancérologie-Biologie-Médecine-Santé (CBMS 582)
- **DOCTORAL SCHOOL:** Université de Paris
- **UNIVERSITY:** Université de Paris
- **FUNCTION:** DR
- **TEL:** (33)1 42 75 42 93
- **E-MAIL:** devillartay@gmail.com

LABORATORY PRESENTATION AND RESEARCH TOPICS

- **SUPERVISOR HDR:** JP de Villartay, devillartay@gmail.com

- **SPECIFY THE TEAM NAME:** Genome Dynamics in the Immune System (DGSI)

- **WEBSITE OF THE TEAM:** https://www.institutimagine.org/fr/DGSIMRH

DESCRIPTION OF THE PROPOSED PROJECT

- **KEYWORDS:** V(D)J recombination, DNA damage and repair, Genome instability, Cancer

ABSTRACT

DNA double strand breaks (DSBs) are the most toxic DNA lesions given their oncogenic potential. Nevertheless, **programmed DSBs (prDSBs)** contribute to several biological processes, such as meiotic recombination, programmed genome rearrangement in *ciliates*, or V(D)J recombination during the development of the adaptive immune system. The co-evolution of processes that couple introduction of prDSBs to their actual repair probably constitutes an effective safeguard against genomic instability.

V(D)J recombination is the molecular process by which exons encoding the variable domain of immunoglobulins and T cell receptors are assembled prior to their expression, thus providing the large diversity of the immune repertoires. It is essentially a "cut and paste" mechanism by which variable (V), diversity (D), and joining (J) segments are associated by a somatic DNA rearrangement process. V(D)J recombination is initiated by a site-specific prDSB introduced by the lymphoid specific **RAG1 and RAG2**. The resulting prDSB is repaired through **Non Homologous End Joining** (**NHEJ)**. A functional redundancy in the DNA repair phase of the reaction between RAG2 and several NHEJ factors appears as a specific control mecanism to avoid genome instability.

Our project is to use V(D)J recombination as a model system to better understand how **coupling of DNA damage and repair** may provide an efficient way of controlling genome stability associated with prDSBs. Several candidate factors have been identified through literature mining, survey of human patients with inherited immune deficiencies as well as a global search for RAG2 protein partners through pull-down and mass spectrometry.

- **DESCRIPTION OF THE PROJECT**

Living organisms are constantly exposed to endogenous and exogenous genotoxic assaults. Highly conserved DNA repair mechanisms have been evolutionary selected to cope with these damages to maintain genome integrity. DNA double strand breaks (DSBs), which represent

the most toxic lesions, are repaired by at least two DNA repair pathways (Homologous recombination and Non Homologous End Joining). Nevertheless, despite their "dangerousness", programmed DSBs (prDSBs) are part of essential physiological processes including meiotic recombination, V(D)J recombination, or genome rearrangements in ciliates [1].

V(D)J recombination is required for the assembly of genes encoding the variable domain of immunoglobulins and T cell receptors, thus ensuring the generation of diversified B and T lymphocyte repertoires [2]. It is essentially a mechanism related to "cut and paste" transposition, in which previously scattered variable (V), diversity (D), and joining (J) segments are physically associated on the DNA by somatic DNA rearrangement. It is initiated by a site specific prDSB introduced by the lymphoid specific, domesticated transposase **RAG1 and RAG2** on recombination signal sequences (RSS) that flank all the rearranging V, D, and J segments [3, 4]. **The repair of RAG1/2 induced DSBs strictly relies on NHEJ**. V(D)J recombination constitutes a critical checkpoint in the development of the adaptive immune system and its default results in **severe immune deficiency (SCID)** both in humans and animal models [5]. Moreover, V(D)J recombination is a potent pro-oncogenic process if not well controlled. Indeed, all animal models that combined NHEJ deficiency on the TP53-/- background result in the early development of **aggressive pro-B cell lymphomas** harboring RAG1/2 driven chromosomal translocations [6].

In previous work, we discovered an efficient two-tier mechanism that ensures the proper repair of RAG1/2 introduced DSBs, thus avoiding genome instability during V(D)J recombination [7]. This mechanism relies on the functional redundancy between the non-core **C-terminus region of RAG2** and the DNA repair factor **Xlf**. Very schematically, only when the two are missing is V(D)J recombination affected resulting in genome instability and the development of lymphomas when introduced on a TP53 KO background. We proposed that this RAG2/Xlf relationship might be at the basis of a **DNA damage/repair coupling "**strategy" that may be generalized to other instances of programmed DSBs [1]. We also demonstrated a functional redundancy between Xlf and PAXX a recently discovered NHEJ factor [8, 9].

In the present PhD proposal, we wish to analyze the molecular basis of DNA damage/repair coupling and its function as a **potent tumor suppressor** by using the V(D)J recombination as a paradigm. V(D)J recombination can be easily analyzed in Abelson immortalized pro-B lymphocytes in culture. The project will follow several aims:

- Analysis of several putative RAG2 binding partners that we identified through a large-scale proteomic study. The interactions will be validated by biochemical and *in cellulo* analyses (proximity ligation assay). The domains of interaction will be determined. The consequences of a loss of these partners on DNA repair will be addressed through i*n cellulo* CRISPR/Cas9 mutagenesis followed by functional complementation with wt. and mutant forms. *In vivo* murine model of gene inactivation (KO or KI) will be developed for the most interesting hits.
- Functional analysis of Ku interacting DNA repair factors involved in the DNA repair phase of V(D)J recombination. Several NHEJ factors contain Ku binding motives (KBM). Genes coding for these factors will be invalidated in Abl B cell lines through CRISPR/Cas9. Resulting mutants will be complemented with various combinations of wt. and mutated form to establish the functional interactome of these factors during the DNA repair phase of V(D)J recombination.
- Transcriptomic analyses of Abl B cells undergoing V(D)J recombination will be conducted through RNAseq to identify the cellular machinery specifically involved in this process.

- We'll take advantage of the powerful readout of V(D)J recombination in Abl B cells to setup a genome wide CRISPR/Cas9 screen to identify proteins critically involved in this process. Recovered candidates will be compared with the list of RAG2 partners as well as with the list of up regulated genes identified through RNAseq.
- We will continue our survey of human patients presenting with immunodeficiency caused defect in V(D)J recombination. To identify these patients we have developed a biomarker based on the study of the T cell repertoire [10].

- **REFERENCES**

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EXPECTED PROFILE OF THE CANDIDATE

- **EXPERIENCE REQUIRED**

Looking for a motivated candidate with theoretical training in Immunology and/or molecular genetics. Lab experience in these domains would be appreciated