



CENTRE DE RECHERCHE EN CANCÉROLOGIE DE LYON

UMR Inserm 1052 - CNRS 5286

Centre Léon Bérard
Cheney D
28 rue Laënnec
69373 LYON Cedex 08

Directeur : Dr. Patrick Mehlen
Secrétariat : Tel : 04 78 78 27 81
Fax : 04 78 78 27 20
claire.couvreur@lyon.unicancer.fr



PROJET DE THESE AVEC ALLOCATION DOCTORALE ANRS

Unité d'accueil : INSERM U1052 – Centre de Recherche en Cancérologie de LYON

Laboratoire d'accueil : Equipe Mécanismes de la pathogénèse de l'hépatite B et C chronique et nouvelles stratégies antivirales

Contact : Dr. Barbara Testoni : barbara.testoni@inserm.fr
Prof. Fabien Zoulim : fabien.zoulim@inserm.fr

Titre du sujet de thèse : Role of the histone chaperone complex HIRA and HBV Core protein in histone deposition on hepatitis B virus minichromosome

Projet de recherche :

Hepatitis B virus (HBV) causes a chronic infection which affects more than 250 million people in the world and is associated to an increased risk of developing severe liver disease, including liver cirrhosis and hepatocellular carcinoma (HCC), the second cause of cancer death worldwide. Therefore, despite the existence of a prophylactic vaccine, HBV remains a global health burden and is classified as the second carcinogen agent in the world, after tobacco.

HBV is a small enveloped DNA viruses, but it could be assimilated to a pararetrovirus, since it requires a mandatory nuclear phase with an RNA intermediate for its replication. Infectious virions contain a partially double-stranded relaxed circular (rc)DNA covalently associated with the viral polymerase protein. To be transcriptionally active, rcDNA has to be converted into a covalently closed circular (ccc)DNA episome in the nucleus of infected hepatocytes. Histones, HBV core (HBc) protein and cccDNA are closely linked together to build a dynamic chromatin structure that translates into different levels of biological activity.

The mechanisms leading to histone deposition onto incoming rcDNA and regulating the histone reshuffling during cccDNA transcription are yet not known. The host laboratory previously demonstrated that the histone chaperone complex HIRA, known to deposit the histone variant H3.3 at site of DNA damage, on naked DNA and in correspondence to active genes, is crucial for the establishment of cccDNA molecules after HBV infection of hepatocytes. Moreover, HIRA silencing severely affects viral RNA levels and HBV replication, suggesting its involvement in the regulation of cccDNA activity once the cccDNA pool is already formed. The HBV core (HBc) protein has been found to be associated both to cccDNA and HIRA and co-immunoprecipitation experiments indicate a possible interaction between the two proteins.

The research project objet of this PhD fellowship proposal, in continuity with the results obtained previously, is aimed at further investigating the role of the histone chaperone complex HIRA in H3.3 deposition on cccDNA and the specific role of the HBV Core protein in this phenomenon. In particular, it will

- characterize the components of the HIRA complex associated cccDNA and to HBc;
- characterize the domains of the HIRA and HBc proteins required for histone deposition on cccDNA;
- investigate the nature of HIRA complex/HBc interaction.

New therapeutic approaches are needed to overcome cccDNA persistence in the infected cells or, at least, to control its transcriptional and replicative activity, since current antivirals are able to cease virion productivity but do not affect either cccDNA reservoir or its activity. The objectives of this fellowship proposal will help in understanding how cccDNA is formed and regulated and might potentially provide a new virus-specific therapeutic strategy to impact both HBV minichromosome formation and activity. In the impossibility to eliminate cccDNA from infected cells, reducing *de novo* infection by blocking cccDNA establishment and controlling its activity would pave the way for long-term control of chronic hepatitis B.

Principales Références :

- (1) Revill PA, Chisari FV, Block JM, Dandri M, Gehring AJ, Guo H, Hu J, Kramvis A, Lampertico P, Janssen HLA, Levrero M, Li W, Liang TJ, Lim SG, Lu F, Penicaud MC, Tavis JE, Thimme R; Members of the ICE-HBV Working Groups; ICE-HBV Stakeholders Group Chairs; ICE-HBV Senior Advisors, **Zoulim F**. A global scientific strategy to cure hepatitis B. *Lancet Gastroenterol Hepatol*. 2019 Jul;4(7):545-558.
- (2) Martinez MG, **Testoni B**, **Zoulim F**. Biological basis for functional cure of chronic hepatitis B. *J Viral Hepat*. 2019
- (3) **Testoni B**, Lebossé F, Scholtes C, Berby F, Miaglia C, Subic M, Loglio A, Facchetti F, Lampertico P, Levrero M, **Zoulim F**. Serum hepatitis B core-related antigen (HBcrAg) correlates with covalently closed circular DNA transcriptional activity in chronic hepatitis B patients. *J Hepatol*. 2019 Apr;70(4):615-625.
- (4) Locatelli M, Fresquet J, Maadadi S, Quivy JP, **Testoni B** and **Zoulim F**. "The histone chaperone HIRA is crucial for the early establishment of hepatitis B virus minichromosome". *Hepatology* (2017) 66, 783A-783A