LabMuse EPIGENMED, project Trans_noise: fully funded PhD studentship

Stochastic fluctuation of HIV-1 transcription: contribution of extrinsic and intrinsic factors to latency exit

Summary of the project: A major surprise of recent live imaging studies is that transcription is often neither continuous, nor deterministic. Even at the steady-state, transcription occurs through periods of activity in which many mRNAs are transcribed in a short time, interspersed with periods of inactivity. This indicates that genes considered active may in fact be randomly alternating between 'ON' and 'OFF' states. In particular, in cells latently infected with HIV-1 the periods of viral expression are brief and rare, rather than totally non-existent. Because of this residual stochastic transcriptional activity, latent viruses can remain at undetectable levels for years and yet rapidly provoke viral rebounds within weeks after interrupting antiviral treatments. Understanding the mechanisms controlling latency is important for developing a HIV cure. Several mechanisms have been proposed to trigger latency exit. Spontaneous activation of the viral promoter occurring because of the stochastic nature of transcription initiation is called intrinsic noise. Other events triggering activation can come from external cues affecting the entire cell and are called extrinsic noise. **In this project, the student will study the stochastic fluctuations of HIV-1 transcription and will separate the contributions of extrinsic and intrinsic factors and their relative importance for viral latency exit.**

Background:

Cells latently infected with HIV-1 prevent patients from being cured. Indeed, latent viruses can remain undetected by the immune system for years and yet rapidly provoke viral rebounds weeks after interrupting antiviral treatments. Understanding the mechanisms controlling latency is important to develop a cure, in particular with the 'Shock-and-Kill' or 'Block-and-Lock' approaches[3]. Latent cells lack the viral activator Tat and are blocked at the level of promoter-proximal polymerase pausing. Several mechanisms have been proposed to trigger latency exit. First, quiescent memory cells carrying a latent virus can be stimulated transiently, for instance by an antigen, and this will activate the viral promoter. These type of events are commonly referred to as extrinsic noise, since they come from external cues affecting the entire cell. Second, spontaneous activation of the viral promoter can also occur due to the inherently stochastic nature of transcription initiation (*ie* intrinsic noise). Interestingly, data from patient cells show that external events and intrinsic noise both contribute to latency exit. For instance, when latent cells from patients are activated with chemicals, only a fraction of the infected cells exits latency, and if the treatment is repeated, another fraction of cells responds[4]. The stochastic fluctuations of viral transcription have been studied in some details, but the respective contributions of extrinsic and intrinsic noise are unknown. Moreover, while RNA imaging approaches provide a direct real-time readout of transcription, they have not been exploited to separate extrinsic from intrinsic noise. This is especially important given that transcriptional noise occurs on multiple time scales, with distinct processes impacting different time scales, and that long time scales predominantly contribute to phenotypic variability[1,2].

Experimental approaches: In this project, the student will study the stochastic fluctuation of HIV-1 transcription and will separate the contribution of extrinsic and intrinsic factors. Extrinsic factors include for instance the cell cycle or spurious activation of pathways controlling viral transcription (NF-kB, NFAT, etc...). To this end, an MS2-tagged HIV-1 reporter[2] will be integrated in multiple independent copies in a reporter cell line (initially as homozygous diploid copies and then as multiple random viral integrations sites). The transcriptional activity of these

copies will be imaged simultaneously as they will form distinct spots in single nuclei. The data will be first analyzed by a pipeline that we previously developed, and which allows to precisely place the timing of each transcription initiation event, for each of these HIV-1 copies. We will then extract the contribution of extrinsic *vs* intrinsic factors by performing various cross-correlation analyses of the viral copies present in the same cell. These analyses will be performed in cells that express the viral activator Tat thus mimicking active cells, as well as in cells lacking Tat and resembling latent cells. Overall, the student will determine the respective importance of extrinsic and intrinsic noise for viral latency exit, with a particular attention on promoter pausing, which is regulated by Tat.

Mathematical modelling approaches:
Models of stochastic transcription resulting from the quantitative analysis of the MS2 signal will be combined with models of population dynamics to generate multiscale models of HIV1 latency exit. The mathematical model of HIV latency will combine two parts: (i) a first part will model the fluctuations of HIV-1 transcription occurring in each single infected cell; (ii) a second part will model the dynamics of the populations of infected cells (proliferation and death of latent cells, reactivation of latent cells leading to newly infected cells, etc. . . . The stochastic transcription model will be reduced with methods previously developed in the Radulescu lab[5,6,7]. The population model will be deterministic. We expect to integrate the two scales in a mesoscopic model. The parameters of the reduced transcription model will be estimated from single cell, single molecule microscopy data for a large panel of latent cells growing in different conditions: normally proliferating, in quiescence or undergoing cellular activation. We expect to characterize the contributions of the intrinsic and extrinsic stochastic variations of HIV-1 promoter activity in the viral rebound after treatment arrest.

References

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Techniques: MS2 imaging, optogenetics; Data analysis (image analysis, statistics, programming in R, Matlab, or Python); Mathematical modelling (continuous- and discrete-time Markov processes).

Candidate: Motivation, perseverance, rigor, creativity and curiosity. Knowledge in HIV biology and mRNA biosynthesis is a plus but not mandatory. Previous training in mathematics and expertise in programming are mandatory. Required diploma: A research master degree from a University, Engineering School or “Grande Ecole”, in a field relevant to the project (biology, mathematics, physics, engineering). Students with interdisciplinary profile are strongly encouraged to apply. *The PhD student will be trained in an interdisciplinary environment and*

his/her contribution will concern all the aspects of the project: theoretical, mathematical and experimental.

Environment: This project belongs to an interdisciplinary PhD training network that will train three students in collaboration with four labs in Montpellier: Edouard Bertrand (IGMM UMR 5535), Ovidiu Radulescu (LPHI UMR 5235), Mounia Lagha (IGMM UMR 5535), and Séverine Chambeyron (IGH UMR 9002). The PhD student will be co-supervised by EB and OR, but will be taught optogenetics in ML lab.The experiments will be primarily performed in the Bertrand lab and the mathematical modelling in the Radulescu lab. The student will join the CBS2 graduate school https://edcbs2.umontpellier.fr/

How to apply: Application files, including a detailed Curriculum Vitae, a motivation letter, diplomas and grades as well as two signed reference letters on headed paper have to be sent to *ovidiu.radulescu@umontpellier.fr*, *edouard.bertrand@igmm.cnrs.fr,* and also uploaded by the candidates to the LabMUSE EpiGenMed website by May 31st, 2020 at the latest, *https://muse.edu.umontpellier.fr/epigenmed-labmuse/*

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