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Host laboratory:
Lab : Institut de Biologie Structurale

Host group/team:
Epigenetics and Molecular Pathways Group

Title of the M2 research internship:
Deciphering bromodomain-mediated control of morphotype switching in *Candida albicans*

Project summary:
Background: Invasive fungal infections are responsible for nearly 4 million deaths annually worldwide, highlighting the urgent need for novel antifungal strategies. *Candida albicans*, a leading fungal pathogen, is a component of the human commensal flora that causes life-threatening invasive infections in immunocompromised individuals. *C. albicans* adapts to diverse host environments by switching between distinct morphotypes. These include a unicellular yeast form optimized for bloodstream dissemination and a filamentous hyphal form responsible for tissue invasion. However, the molecular basis of morphotype switching – a key determinant of pathogenicity – remains poorly understood. Our team has identified the bromodomains (BDs) of Bdf1, a member of the BET family of transcriptional regulators, as promising antifungal targets. BET BDs associate with chromatin by binding histones bearing a key post-translational modification (PTM), acetylation, on specific lysine residues. Bdf1 BDs are essential for *C. albicans* viability and have a wider ligand-binding pocket than their human BET homologs, allowing selective inhibition by small molecules. In a recent breakthrough, we discovered that the wider pocket of *Candida* BDs enables them to recognize bulkier histone PTMs, such as butyrylation and crotonylation. Exposure to butyrate (a gut metabolite) or crotonate (a metabolite encountered in the macrophage) drives *C. albicans* into the yeast form, whereas a "humanized" strain, in which the *Candida* BDs are replaced by their human counterparts, fails to undergo this switch. This finding suggests a crucial role for Bdf1 BDs in morphotype determination and the metabolic plasticity that contributes to *C. albicans* virulence.

Objectives: This project aims to define the role of Bdf1 BDs in *C. albicans* morphotype switching. Specific goals of this M2 internship are to: 1) demonstrate that Bdf1 BDs recognize histone crotonylation in the cell nucleus; and 2) uncover the molecular determinants that enable *Candida* Bdf1 BDs to recognize bulky PTMs.

Methodology: Task 1. Investigating Bdf1 mobility in response to chromatin crotonylation. This task will determine whether *C. albicans* Bdf1 BDs recognize crotonylated histones in a cellular context. Our preliminary FRAP (fluorescence recovery after photobleaching) experiments in HEK cells expressing GFP-tagged *Candida* Bdf1 revealed a decrease in nuclear mobility upon treatment with a chemical inhibitor that enhances histone acetylation. A similar effect was observed for the human BET ortholog Brd4, and mobility in both cases was restored with the BD inhibitor JQ1, confirming BD-mediated chromatin binding. We hypothesize that *Candida* Bdf1, but not human Brd4, will exhibit reduced mobility when histone crotonylation is increased. To test this, we will treat cells with crotonate to enhance histone crotonylation and assess Bdf1 and Brd4 nuclear mobility using FRAP. To confirm that effects are BD-mediated, we will examine the mobility of humanized Bdf1 (where *Candida* BDs are replaced by human BDs) and "candidized" Brd4 (where human BDs are replaced by *Candida* BDs). Experiments will be performed in both human and fungal cells.

Task 2. Structural basis of bulky PTM recognition by Bdf1 BDs. This task will determine how *C. albicans* Bdf1 BDs recognize bulky histone PTMs such as crotonylation and butyrylation. We will recombinantly express and purify Bdf1 BDs using a well-established protocol and incubate them with crotonylated or butyrylated

histone peptides for crystallization trials. Diffraction data will be collected at the neighboring ESRF synchrotron, and structures will be solved using molecular replacement. To validate our findings, we will introduced targeted mutations in *Candida* and human BDs to swap residues critical for ligand selectivity. Histone peptide binding assays will assess whether these mutations alter PTM recognition, further defining the structural basis of bulky PTM selectivity.

Techniques: This project will provide hands-on training in: (i) fluorescence microscopy and FRAP analysis of human and fungal cells; (ii) protein purification and biochemical binding assays; (iii) X-ray crystallography and structural analysis.

Expected Outcome: This study will provide new insights into how *C. albicans* adapts to host environments by regulating morphotype switching through bromodomain-PTM interactions. The project is well-suited for highly motivated M2 students with a strong interest in epigenetics, structural biology and/or host-pathogen interactions. Prior experience in microscopy, protein biochemistry, or structural methods is beneficial but not required.

Keywords:
Invasive fungal infection, *Candida*, epigenetics, host-pathogen interactions, antifungal target

Relevant publications of the team:

1. Cooperative binding of two acetylation marks on a histone tail by a single bromodomain.
Morinière J, Rousseaux S, Steuerwald U, Soler-López M, Curtet S, Vitte A-L, Govin J, Gaucher J, Sadoul K, Hart DJ, Krijgsveld J, Khochbin S, Müller CW, **Petosa C.**
Nature 2009, 461:664-8. https://doi.org/10.1038/nature08397
2. Bromodomains: structure, function and pharmacology of inhibition.
Ferri E, **Petosa C**, McKenna CE.
Biochem Pharmacol 2016, 106:1-18. https://doi.org/10.1016/j.bcp.2015.12.005
3. Selective BET bromodomain inhibition as an antifungal therapeutic strategy.
Mietton F, Ferri E, Champleboux M, Zala N, Maubon D, Zhou Y, Harbut M, Spittler D, Garnaud C, Chauvel M, d'Enfert C, Kashemirov BA, Hull M, Cornet M, McKenna CE*, Govin J*, **Petosa C***.
Nature Commun 2017, 8:15482. https://doi.org/10.1038/ncomms15482
4. A new hope to fight invasive fungal infection. [French]
Petosa C, Govin J, Mietton F.
Med Sci 2018, 34:123-125. https://doi.org/10.1051/medsci/20183402007
5. Towards More Potent Imidazopyridine Inhibitors of *Candida albicans* Bdf1: Modeling the Role of Structural Waters in Selective Ligand Binding.
Zhou Y, Overhulse JM, Dupper NJ, Guo Y, Kashemirov BA, Wei K, Govin J, **Petosa C,** McKenna CE.
J Comput Chem 2022, 43:2121-2130. https://doi.org/10.1002/jcc.26997
6. Humanized *Candida* and NanoBiT Assays Expedite Discovery of Bdf1 Bromodomain Inhibitors with Antifungal Potential. Wei K, Arlotto M, Overhulse JM, Dinh T-A, Zhou Y, Dupper NJ, Yang J, Kashemirov BA, Dawi H, Garnaud C, Bourgine G, Mietton F, Champleboux M, Larabi A, Hayat Y, Indorato RL, Noirclerc-Savoye M, Skoufias D, Cornet M, Rabut G, McKenna CE*, **Petosa C***, Govin J*.
Advanced Science 2025, Jan 16:e2404260. https://doi.org/10.1002/advs.202404260