ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by: Claudio Alfieri

Project details

Project title: Molecular investigation on MuvB oncogenic complexes

Supervisory team

Primary Supervisor: Claudio Alfieri

Associate Supervisor(s): Jörg Mansfeld

Secondary Supervisor: Rob Van Montfort

Divisional affiliation

Primary Division: Division of Structural Biology

Primary Team: Molecular Mechanisms of Cell Cycle Regulation

Site: Chester Beatty Laboratories

Project background

The cell cycle is the fundamental biological process where the cell coordinates chromosome replication and segregation with cell growth and division.

This interdisciplinary project which combines structural and cell biology, focuses on dissecting the molecular mechanisms of cell cycle regulation mediated by macromolecular complexes.

The Alfieri lab is specialised in studying these complexes [1-5] by using a combination of protein complex reconstitution, biochemical analysis, structural characterization by cryo-EM and functional analysis in cell lines.

Disruption of the cell cycle is one of the main features of cancerous cells. Understanding the molecular mechanism of cell cycle regulation will contribute to (i) understanding the fundamental process of the cell cycle and to (ii) designing novel anti-cancer drugs to be used in more effective therapies targeting both cycling and quiescent tumour cells.

Project aims

- Biochemical and biophysical characterisation of protein complexes
- Structural analysis and design/generation of structure-based mutants
- Functional characterization of the mutants with purified components and in cells

Research proposal

Synchronised expression of cell cycle genes requires the action of specific transcriptional regulators which recognise cell cycle genes promoters and drive their cell cycle dependant transcription.

A key regulator of cell cycle dependant transcription that we study in my lab is the evolutionary conserved five-subunit (i.e. RbBP4, LIN9, LIN37, LIN52 and LIN54) protein complex named **MuvB** and its associated factors.

MuvB is a remarkably interesting transcriptional regulator because of its peculiar **dual function as a transcriptional activator and repressor**. This “yingyiang” activity of MuvB is crucial for both cell cycle exit and cell proliferation. MuvB represses around 1000 genes during a cell cycle arrest and by changing associated factors and post-translational modifications, it switches from a transcriptional repressor to an activator during the G2/M cell cycle phase transition in proliferating cells.

The key goal of our research program is **to understand how the transcriptional regulator MuvB switches from repressor to activator and how this impacts cellular proliferation decisions within the cell cycle and in cancer.**

To accomplish this, our research plan lies at the crossroads between two main pillars of research involving (i) experiments with purified native and recombinant protein complexes and (ii) experiments in cells. We aim to identify and analyse interactions of MuvB with associated factors and complexes with enzymatic activity, and post-translational modifications (PTMs) that accompany the MuvB switch. We will study in detail how these (i) influence MuvB engagement of target chromatin and how they (ii) influence cell proliferation, cell cycle phases, and MuvB subcellular localization, and rationalize alterations found in cancer. Combining highly detailed molecular information with system-wide information is key for designing the anti-cancer drugs of the future.

MuvB is a repressor when bound to the retinoblastoma-like (RBL) proteins during a cell cycle arrest, and it is unclear how it switches to an activator once it dissociates from the RBL proteins and recruits the B-MYB and FOXM1 oncoproteins during cell proliferation. We therefore plan to study how MuvB assembles with associated factors to uncover the MuvB activator/repressor switch mechanism.

This specific project focuses on studying interactions between MuvB and transcriptional activators including the MYB and FOXM1 oncogenes.

We can already reconstitute complexes of MuvB with B-MYB and FOXM1. Stoichiometry and stability of these complexes will be characterized by mass photometry. The binding affinity between these interaction partners will be measured by isothermal titration calorimetry (ITC) and/or fluorescence polarization (FP). Within these experimental setups, competition experiments between subunits that bind in overlapping sites on MuvB will be performed. With these complexes in hand, we will perform a structural analysis by using cryo-electron microscopy (cryo-EM) and XL-MS. Using knockout cell lines, we will assess the effect of structure-based mutations coming from our structural and biochemical data, by performing rescue experiments with different MuvB mutant genes in cells.

Outcomes

Characterization of MuvB interactions with oncogenic proteins will aid our molecular investigation on the MuvB pathway in cancer cell proliferation. This will allow us to design structure-based mutants and post-translational modification site mutants that will affect gene activating functions of MuvB to test in cells, thereby elucidating the mechanism of how these components cooperate to establish the dual-function MuvB transcriptional switch which governs cell-cycle dependent gene regulation and cell proliferation decisions. The framework of interactions unveiled will also inform on how activating and repressive interactions are mutated in cancer and how these may contribute to cancer progression and relapse.

This work will also pave the basis for the design of new anti-cancer drugs targeting the MuvB switch in cancer cells that rely on MuvB for escaping anti-cancer therapy.

Literature references

[1] ALFIERI, C., CHANG, L. & BARFORD, D. 2018. Mechanism for remodelling of the cell cycle checkpoint protein MAD2 by the ATPase TRIP13. *Nature,* 559**,** 274-278.

[2] ALFIERI, C., CHANG, L., ZHANG, Z., YANG, J., MASLEN, S., SKEHEL, M. & BARFORD, D. 2016. Molecular basis of APC/C regulation by the spindle assembly checkpoint. *Nature,* 536**,** 431-436.

[3] ALFIERI, C., TISCHER, T. & BARFORD, D. 2020. A unique binding mode of Nek2A to the APC/C allows its ubiquitination during prometaphase. *EMBO Rep,* 21**,** e49831.

[4] FAN, X., WANG, Y., JIANG, T., LIU, T., JIN, Y., DU, K., NIU, Y., ZHANG, C., LIU, Z., LEI, Y. & BU, Y. 2021. B-Myb accelerates colorectal cancer progression through reciprocal feed-forward transactivation of E2F2. *Oncogene,* 40**,** 5613-5625.

[5] KOLIOPOULOS, M. G., MUHAMMAD, R., ROUMELIOTIS, T. I., BEURON, F., CHOUDHARY, J. S. & ALFIERI, C. 2022. Structure of a nucleosome-bound MuvB transcription factor complex reveals DNA remodelling. *Nat Commun,* 13**,** 5075.

Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants:	Candidates must have a first class or upper second-class honours BSc Honours/MSc in Biology, Biochemistry or Molecular/Cell biology, and proven experience in wet lab work. Candidates must have interest in cell cycle and experience in cell biology. Experience in protein biochemistry and/or structural biology would also be desirable.
Intended learning outcomes:	• The student will work in a high-quality structural and cell biology laboratory and will be exposed to all the practical challenges that are often encountered in these disciplines. • Ability to critically read and understand scientific articles relevant to the project. • Ability to present the scientific results to appropriate scientific conferences. • Ability to write high-quality scientific manuscripts.

Advertising details

Project suitable for a student with a background in:

- [x] Biological Sciences
- [ ] Physics or Engineering
- [ ] Chemistry
- [ ] Maths, Statistics or Epidemiology
- [ ] Computer Science