ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	ICR

Project details

Project title:	Structural and functional studies of the regulation of mRNA splicing by cyclin-dependent kinases

Supervisory team

Primary Supervisor:	Basil Greber
Associate Supervisor(s):	TBC
Secondary Supervisor:	Vlad Pena

Divisional affiliation

Primary Division:	Structural Biology
Primary Team:	Structural Biology of DNA Repair Complexes
Site:	Chelsea

Project background

Cyclin-dependent kinases

Human cells contain approximately 20 different members of the cyclin-dependent kinase (CDK) family, the activities of which depend on accessory cyclin subunits. Some CDKs exclusively associate with a single type of cyclin, while others can associate with a variety of different cyclins, providing a mechanism to further regulate their function and substrate specificity (Malumbres, 2014).

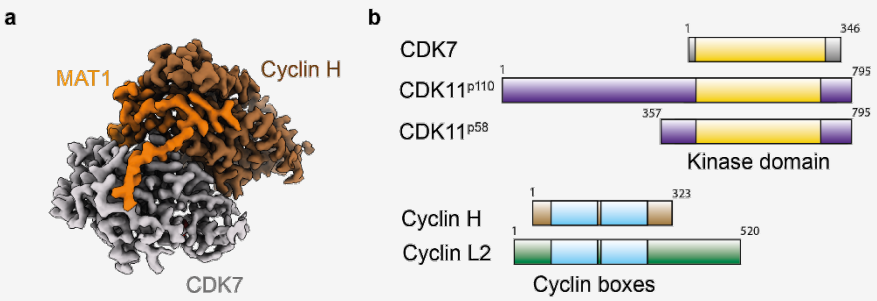


Figure 1 – CDK-cyclin complexes studied in this proposal. (a) Cryo-EM map of the human CAK coloured by its constituent subunits (Greber et al., 2020). (b) Domain architectures of CDK7, CDK11 and their associated cyclins H and L2.

Many CDKs play critical roles in controlling the events of the cell cycle, while others, including CDKs 7, 8, 9, 11, 12, and 13, exclusively or additionally regulate events in transcription (Harlen and Churchman, 2017). Due to their important functions, many CDKs, including CDK7 and CDK11, have been identified as targets for cancer therapy (Lin et al., 2019, Sava et al., 2020). Notably, high-resolution structures from the Greber team have provided avenues towards structure-based drug design for CDK7 (Greber et al., 2020).

Cyclin-dependent kinases interact with splicing factors

There is increasing evidence for additional roles of CDKs in the regulation of mRNA splicing, a possible way for coordination of splicing with transcription (Loyer and Trembley, 2020). CDK11 phosphorylates SF3B1, thereby regulating spliceosome activation (Hluchý et al., 2022), and it associates with splicing regulator (SR) proteins (Loyer and Trembley, 2020, Loyer et al., 2008). Similarly, cyclin H, the partner cyclin of CDK7, associates with complexes that contain the U1 snRNA, suggesting that the CDK7-cyclin H-MAT1 complex (CAK) interacts with the U1 snRNP (O'Gorman et al., 2005). These observations raise important mechanistic questions that this PhD studentship project aims to address.

Project aims

- Purification and structure determination of CDK11-cyclin L2
- Pull-down and mass-spectrometric identification of splicing components in nuclear extracts using CAK and CDK11-cyclin L2 as baits
- Structure determination of CDK-splicing complexes
- Structure-based mutagenesis to validate structural findings

Research proposal

PROJECT OVERVIEW

The overarching aim of this PhD studentship project is to study how CDK7-cyclin H-MAT1 (CAK) and CDK11-cyclin L2 interact with splicing components to investigate the function of CDKs at the interface between transcription and splicing. The interactions of both CDK-cyclin complexes with splicing components are documented in the literature (O'Gorman et al., 2005, Loyer et al., 2008, Hluchý et al., 2022), though important mechanistic questions remain. These will be addressed in this project. Among the primary subjects of the proposed work, CDK7 is very well characterised with extensive functional data and high-resolution structures available (Fisher, 2019, Greber et al., 2020), while CDK11 is less-well studied, and no structures are available (Loyer and Trembley, 2020). Both CDKs have been implicated in cancer (Loyer and Trembley, 2020), and drug discovery programmes are underway (Sava et al., 2020, Lin et al., 2019).

The project is to be hosted as a collaborative endeavour in the laboratories of Dr. Basil Greber, an expert in high-resolution cryo-electron microscopy (cryo-EM) of CDKs, and Prof. Dr. Vlad Pena, an expert in the structural biology of splicing.

AIMS OF THE PROJECT

Aim 1: What is the structure of of CDK11-cyclin L2?

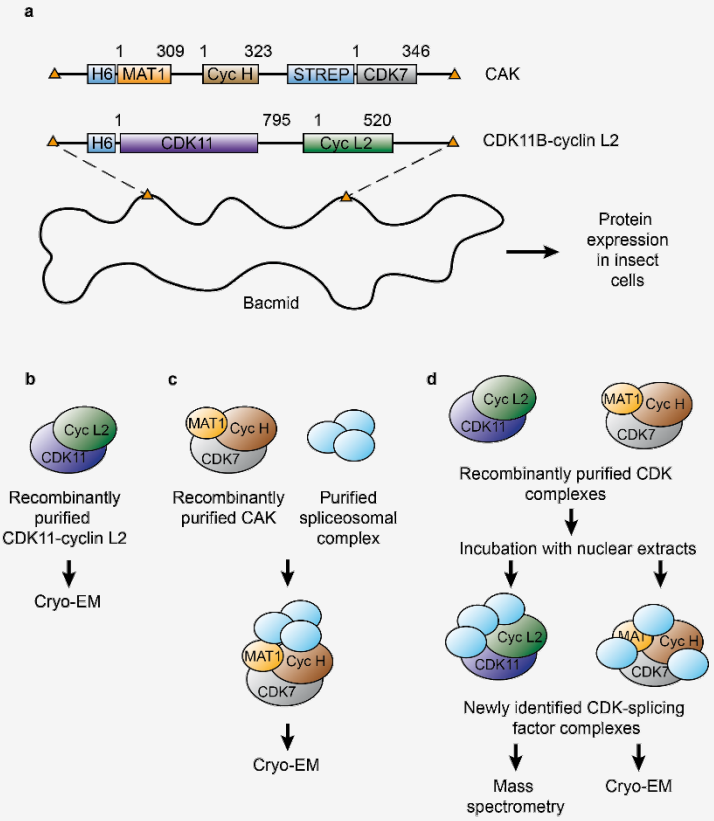
Aim 1.1: Reconstitution of biochemically pure CDK11-cyclin L2 complexes

The purification and structure determination of CAK has been established in the Greber team (Greber et al., 2020). However, no structures of CDK11-cyclin L complexes exist. Interestingly, both CDK11 and its cyclins are larger than the typical CDK-cyclin pair, with the largest isoform of CDK11, termed CDK7p110, weighing in at approximately double the molecular weight compared to transcriptional CDKs such as CDK7 or CDK9. Additional isoforms exist, including CDK11p58, an approx. 50 kDa fragment generated by alternative translation initiation.

The student will learn how to express CDK11-cyclin L2 in insect cells from codon-optimised genes in the dedicated facility of the Division of Structural Biology and will purify the complex using standard techniques (affinity and size exclusion chromatography), using the methods established for CDK7-cyclin H-MAT1 (CAK) as a starting point. Both the p110 and p58 isoforms of CDK11 will be produced (Fig. 2a).

This phase of the project will allow the student to learn or consolidate basic molecular biology techniques and become fully independent in their application.

Figure 2 – Outline of the project. (a) Recombinant expression of CDK-cyclin complexes in insect cells using bacmids. (b) Structure determination of CDK11-cyclin L2. (c) Reconstitution of complexes from purified components. (d) Isolation of new complexes using purified CDK-cyclin complexes as baits.



Aim 1.2: Structure determination by cryo-EM

Due to the domain organisation of CDK11-cyclin L2 (Fig. 1b), which both comprise extended unstructured segments, crystallisation for structure determination by X-ray diffraction is not a promising avenue for structure determination of full-length CDK11-cyclin L2 complexes. Therefore, the student will determine the structure of the CDK11-cyclin L2 complex using cryo-EM on the Glacios cryo-transmission electron microscope at the ICR, based on the results of aim 1.1 (Fig. 2b). Image processing will be performed on available high-performance servers that leverage graphics processing units (GPUs).

If required, as judged by the outcome of initial structure determination, truncations can be designed if required to increase the resolution of the core components, including the active site and substrate binding sites of CDK11-cyclin L2, which may provide avenues towards structure-based drug design in this system (an application that is, however, not a primary goal of the PhD studentship).

This stage of the project will provide the student with an opportunity to develop the skills required for structure determination of molecular complexes by cryo-EM. The student will be presented with further opportunities to expand these skills on more challenging targets under aim 2.

Aim 2: How do CAK and CDK11-cyclin L2 interact with splicing factors?

Aim 2.1: Pull-down and mass-spectrometric identification of splicing components in nuclear extracts using CAK and CDK11-cyclin L as baits

Using purified CDK11-cyclin L2 (aim 1) or purified CAK (already established), both containing affinity tags such as GST, FLAG, or twin-STREP, the student will characterise the interactome of these kinases in nuclear extracts and against partially or fully purified spliceosomal complexes (Fig. 2c, d). The required methods have been established in the laboratory of Prof. Vlad Pena.

Bait proteins will be incubated in nuclear extracts or with purified spliceosomal complexes and then isolated using their respective affinity tags, followed by sucrose gradient ultracentrifugation for verification of the interactions and to separate different complexes according to their size. The resulting complexes will be analysed by mass-spectrometry to identify new interactors and confirm previously reported interactions.

If stable interactions with previously characterised spliceosomal assemblies are detected, chemical crosslinking combined with mass spectrometry will be used to provide a first insight into the global architecture of the complexes, based on the framework provided by already-existing structures of spliceosomal assemblies.

Aim 2.2: Structure determination of CDK-splicing complexes

The student will subject the complexes obtained in aim 2.1 as well as in vitro-assembled complexes of CAK and the U1 snRNP to structure determination by cryo-EM. If required, methods to stabilise complexes, such as crosslinking with glutaraldehyde or BS3, will be exploited. Initial interpretation of lower-resolution densities will leverage libraries of AlphaFold models curated based on mass-spectrometric inventories (aim 2.1) and distance restraints from crosslinking mass-spectrometry experiments (aim 2.1); high-resolution three-dimensional reconstructions will be interpreted by full atomic models. These will allow deduction of new interaction interfaces and critical functional residues.

This stage of the project will allow the student to extend their skills in cryo-EM and apply more advanced image processing methods to these more complex assemblies.

Aim 3: How do the newly identified interaction interfaces contribute to functional mechanisms?

Functional consequences of mutations to residues critical for complex formation, as deduced from the results of aim 2.2, will be investigated in cellular assays to test effects on cell growth and viability. Additionally, cellular mRNA will be profiled to detect changes to mRNA levels or changes in abundance of specific isoforms due to disruption of processing complexes.

OUTCOMES AND IMPACT

The research proposed here aims to uncover important mechanistic data that will substantially expand our understanding of the crosstalk between CDKs, transcription, and splicing.

Literature references

[1] FISHER, R. P. 2019. Cdk7: a kinase at the core of transcription and in the crosshairs of cancer drug discovery. Transcription, 10, 47-56.

[2] GREBER, B. J., PEREZ-BERTOLDI, J. M., LIM, K., IAVARONE, A. T., TOSO, D. B. & NOGALES, E. 2020. The cryoelectron microscopy structure of the human CDK-activating kinase. Proc. Natl. Acad. Sci. USA, 117, 22849-22857.

[3] HARLEN, K. M. & CHURCHMAN, L. S. 2017. The code and beyond: transcription regulation by the RNA polymerase II carboxy-terminal domain. Nat. Rev. Mol. Cell. Biol., 18, 263-273.

[4] HLUCHÝ, M., GAJDUŠKOVÁ, P., MOZOS, I. R. D. L., RÁJECKÝ, M., KLUGE, M., BERGER, B.-T., SLABÁ, Z., POTĚŠIL, D., WEIS, E., ULE, J., ZDRÁHAL, Z., KNAPP, S., PARUCH, K., FRIEDEL, C. C. & BLAZEK, D. 2022. CDK11 regulates pre-mRNA splicing by phosphorylation of SF3B1. Nature, 609, 829-834.

[5] LIN, A., GIULIANO, C. J., PALLADINO, A., JOHN, K. M., ABRAMOWICZ, C., YUAN, M. L., SAUSVILLE, E. L., LUKOW, D. A., LIU, L., CHAIT, A. R., GALLUZZO, Z. C., TUCKER, C. & SHELTZER, J. M. 2019. Off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials. Science Translational Medicine, 11, eaaw8412.

[6] LOYER, P. & TREMBLEY, J. H. 2020. Roles of CDK/Cyclin complexes in transcription and pre-mRNA splicing: Cyclins L and CDK11 at the cross-roads of cell cycle and regulation of gene expression. Semin. Cell Dev. Biol., 107, 36-45.

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[8] MALUMBRES, M. 2014. Cyclin-dependent kinases. Genome Biol., 15, 122.

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:	BSc or MSc in biochemistry, molecular biology, or structural biology
Intended learning outcomes:	• Expression of human protein complexes in insect cells • Protein purification using various techniques • Interaction studies in vitro and using cell lysates/nuclear extracts

- Structure-determination by cryo-electron microscopy, including data collection, data processing, model building, and interpretation
- Writing of 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