ICR The Institute of Cancer Research

CANCER RESEARCH UK

PhD Project Proposal

Funder details

Studentship funded by:	Cancer Research UK

Project details

Project title:	Structural dissection of β-catenin ubiquitylation by cryo-electron microscopy

Supervisory team

Primary Supervisor:	Professor Sebastian Guettler
Associate Supervisor(s):	Dr Vasundara Srinivasan
Secondary Supervisor:	Dr Claudio Alfieri

Divisional affiliation

Primary Division:	Structural Biology
Primary Team:	Structural Biology of Cell Signalling (Sebastian Guettler)
Site:	Chelsea

Project background

The Wnt/β-catenin signalling pathway is a fundamental regulator of embryonic development, tissue homeostasis, and regeneration[1]. Central to this pathway is the β-catenin destruction complex (DC), a multi-protein assembly that orchestrates the phosphorylation and subsequent ubiquitylation of β-catenin, earmarking it for proteasomal degradation (Figure 1A)[2,3]. Dysregulation of the DC is a hallmark of several cancers, most notably colorectal cancer (CRC), where mutations in the tumour suppressor adenomatous polyposis coli (APC) and the proto-oncogene β-catenin are prevalent. Disruptions in axis inhibition protein 1 (AXIN1), the DC's central scaffolding subunit, are observed in hepatocellular and endometrial carcinomas[4,5].

Despite its clinical relevance, the Wnt/β-catenin pathway remains untargeted by approved therapeutics. This is largely due to (mostly stem-cell directed) toxicity associated with pathway inhibition, the pathway's mechanistic complexity, and a lack of structural/mechanistic insights into its regulation to reveal novel targeting opportunities[6]. A deeper understanding of the molecular architecture and regulation of the complexes controlling the pathway, including the DC, is essential to both understand fundamental molecular mechanisms and identify novel therapeutic nodes.

The DC's scaffolding proteins APC and AXIN1 co-recruit β-catenin along with casein kinase 1 (CK1) and glycogen synthase kinase 3β (GSK3β). These kinases sequentially phosphorylate β-catenin at a phosphodegron, thereby enabling β-catenin's recognition by the $SCF^{\beta\text{-TrCP}}$ E3 ubiquitin ligase complex[7], which gives rise to polyubiquitylation and subsequent proteasomal degradation of β-catenin[2,3].

Recent work in the laboratory has led to the successful reconstitution of the DC from purified proteins and recapitulated that AXIN1 self-assembly and APC are critical, both for efficient β-catenin phosphorylation and ubiquitylation[8]. Notably, we discovered a direct interaction between APC and $SCF^{\beta\text{-TrCP}}$, suggesting a previously unappreciated regulatory role for APC in modulating β-catenin degradation. However, the structural basis and functional consequences of this interaction remain unknown. Likewise, other potential interaction points between the DC and $SCF^{\beta\text{-TrCP}}$, underpinning the formation of a potential super-complex, remain unexplored[9–13].

This proposal seeks to dissect the structural details of mechanisms governing β-catenin ubiquitylation within the DC using cryo-electron microscopy (cryo-EM), complemented by biochemical and proteomics approaches. The central hypotheses are (1) that the architecture of the DC:$SCF^{\beta\text{-TrCP}}$ super-complex directs ubiquitylation site choice, with consequences for how β-catenin is corralled through the complex, and (2) that APC, and potentially AXIN1, not only scaffold the DC but also facilitate the communication between the DC and $SCF^{\beta\text{-TrCP}}$ for efficient β-catenin processing. An in-depth structural characterisation of these complexes will illuminate whether and how APC truncations, which occur in CRC, dysregulate the normal dialogue between the complexes.

The candidate, funded by Cancer Research UK, will join a multidisciplinary, supportive team dedicated to investigating the mechanisms of normal and oncogenic Wnt/β-catenin signalling. The project will provide extensive training in biochemistry, biophysics and single-particle cryo-EM, with opportunities to gain experience in proteomics (including structural proteomics) through collaborations.

Project aims

- Determine cryo-EM structures of $SCF^{\beta\text{-TrCP}}$ in complex with components of the DC, paired with structural proteomics
  → Reveal how DC components, particularly APC, interact with the β-catenin ubiquitylation machinery
- Visualise substrate-engaged states of $SCF^{\beta\text{-TrCP}}$
  → Capture intermediates to elucidate how β-catenin is positioned for ubiquitylation
- Integrate cryo-EM with biochemical assays to map functional interfaces
  → Identify key contact points and elucidate how they regulate β-catenin modification and turnover
- Assess consequences of APC truncation on complex assembly
  → Understand whether and how cancer-associated mutations alter β-catenin ubiquitylation

Research proposal

Our recent work has shown that APC directly interacts with SCF$^{\beta\text{-TrCP}}$ and promotes β-catenin ubiquitylation, modulating processivity[8], but the structural basis of this interaction and its precise mechanistic impact remain unknown. We also lack structural insights into how other components of the DC, most notably AXIN1, may facilitate the dialogue with the ubiquitylation machinery[9–13]. Over the last decade, cryo-EM has transformed our ability to resolve the architecture of large, dynamic protein complexes. This project aims to use cryo-EM to dissect the structural mechanisms of β-catenin ubiquitylation in the context of a DC:SCF$^{\beta\text{-TrCP}}$ super-complex.

Project Objectives

1. Investigating the APC:SCF$^{\beta\text{-TrCP}}$ complex

To investigate how APC promotes β-catenin ubiquitylation, the candidate will study the structure of APC:SCF$^{\beta\text{-TrCP}}$ complex variants using single-particle cryo-EM. This will entail target expression in the baculovirus-insect cell system and the subsequent purification and assembly of complexes (Figure 1B). Prior to structural characterisation, the relevant complexes will be analysed by biophysical techniques, including size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) and mass photometry.

The candidate will next turn to characterising the complexes by EM. Initial explorations by negative-stain EM will be followed by cryo-EM, which offers opportunities to build experience in dealing with common challenges such as maintaining sample integrity at the air-water interface and preferred orientation[14]. Following hands-on or remote data collection, using the in-house Glacios TEM, or Titan Krios TEMs available through the London Consortium for Cryo-EM (LonCEM) or the Electron Bio-Imaging Centre (eBIC) at the Diamond Light Source, the candidate will gain extensive experience in cryo-EM data processing, structural model building and interpretation. This work will be supported by Team Members and Facility Managers. Opportunities for parallel, complementary structural proteomics studies (by crosslinking mass spectrometry and hydrogen-deuterium exchange mass spectrometry[15]), in collaboration with experts in these areas, are available.

Structural insights into APC:SCF$^{\beta\text{-TrCP}}$ interactions will guide site-directed mutagenesis to modulate identified binding interfaces. The candidate will next probe the functional relevance of these interfaces through *in vitro* β-catenin phosphorylation and ubiquitylation assays with the reconstituted DC[8] (Figure 1C).

2. Expanding the complex to understand the mechanism of β-catenin ubiquitylation

Accompanying the characterisation of the APC:SCF$^{\beta\text{-TrCP}}$ complex, the candidate will build up the assemblies further, to explore the mechanistic details of β-catenin ubiquitylation. Additional components will include NEDD8 on CUL1[16], E2 ubiquitin conjugating enzymes (e.g., UBCH5, CDC34), full-length phosphorylated β-catenin, and ubiquitin. Established approaches to trap transient states for subsequent structural studies by cryo-EM and structural proteomics will be used[17]. Structural studies will be followed by site-directed mutagenesis and functional studies *in vitro*, as outlined above. The candidate will collaborate with Professor Jyoti Choudhary (Functional Proteomics Group and ICR Proteomics Core Facility) to map ubiquitylation sites within β-catenin by mass spectrometry. Studying structure-based mutant variants of the system will enable the candidate to relate ubiquitylation complex architecture to ubiquitylation site specificity.

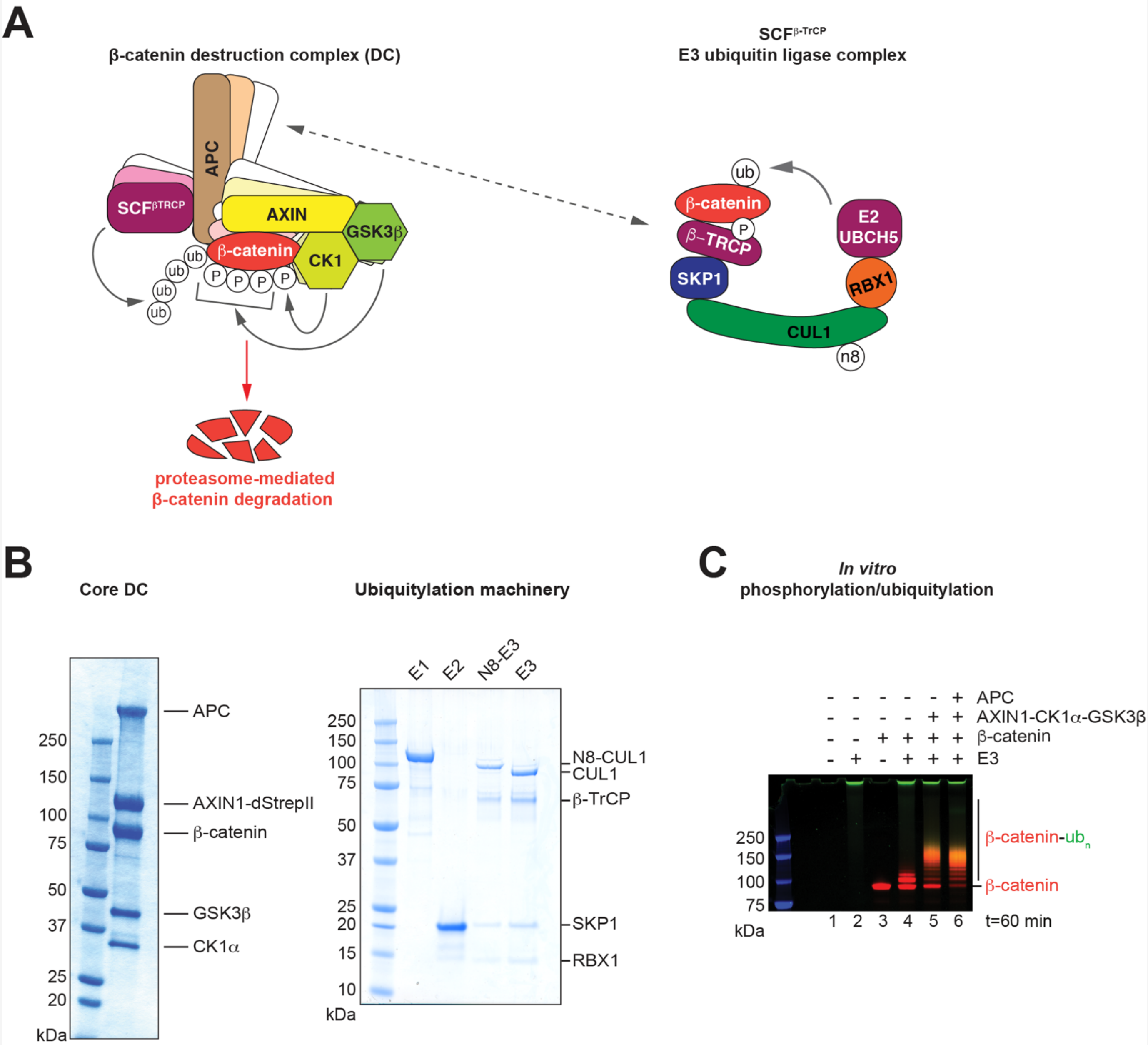

Figure 1: Mechanism and function of the APC:SCF$^{β\text{-}TrCP}$ interaction.

(A) Schematic representation of the DC and SCF$^{β\text{-}TrCP}$. **(B)** SDS-PAGE/Coomassie analysis of components of the DC and ubiquitylation machinery (E1 ubiquitin-activating enzyme UBA1, E2 ubiquitin-conjugating enzyme UBCH5C, E3 composed of CUL1, β-TrCP, SKP1 and CUL1 and its NEDDylated (N8) variant). **(C)** Fluorimetric *in vitro* ubiquitylation assay for β-catenin. Ubiquitin is labelled with FITC, β-catenin with Alexa Fluor 680. Images were adapted from reference 8.

Perspective and Impact

This project will provide the first high-resolution structural insights into β-catenin ubiquitylation within the wider DC:SCF$^{β\text{-}TrCP}$ system. By elucidating how APC mediates the communication between the DC and the E3 ligase and how full-length β-catenin is engaged, the work will advance our understanding of Wnt/β-catenin signalling and its dysregulation in cancer. We anticipate that insights will inform novel strategies to modulate β-catenin stability, eventually opening up new avenues for targeted cancer therapy.

Literature references

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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, Biophysics or Structural Biology with demonstrable laboratory experience
Intended learning outcomes:	• Mastery of single-particle cryo-EM (sample preparation, data collection, processing, model building and interpretation) • Expertise in protein biochemistry/biophysics (expression, purification, reconstitution, binding assays, *in vitro* phosphorylation and ubiquitylation, SEC-MALS, mass photometry) • Skills in proteomics sample preparation and data analysis • Expertise in Wnt/β-catenin signalling and knowledge of cancer biology • Cross-disciplinary data integration from structural mechanisms to cellular phenotypes

Advertising details

Project suitable for a student with a background in:

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