



Lincoln's Inn Fields

Protein Structure Function

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Since its discovery in 1980, post-translational modification of proteins by ubiquitin has been found to be involved in virtually every cellular process, from cell-cycle control to the wound response in plants. Much of the early work focussed on the observation that ubiquitin was required for proteasomal degradation of proteins and that a polyubiquitin modification of 4 or more ubiquitin molecules linked via lysine 48 would target the modified substrate for destruction by the proteasome. However, in more recent years it has become apparent that ubiquitin is not simply a death tag. There are incidences of mono-ubiquitination, multiple monoubiquitination, and a variety of branched poly-ubiquitin modifications including but not limited to K63- and K48-linked chains, and there is now ample evidence that diverse fates await the modified substrate depending on its type of modification. Importantly, ubiquitination plays a central role in cancer biology, as modification of target proteins is a signal for DNA repair, entry into different phases of the cell-cycle, and regulation of tumour suppressors.

Regulation of ubiquitination

Given the complexity of the system it is unsurprising that there are many proteins involved in the regulation of ubiquitin modification. Ubiquitination is achieved through a hierarchical cascade of enzymes: E1, which activates Ub in an ATP-dependent manner; E2, which forms a thioester linkage with Ub; E3 which mediates the transfer of ubiquitin from E2 to the target, and therefore provides the substrate specificity. Many E3 enzymes are involved in disease states, particularly cancer, as their pivotal role in selection of substrate and mediation of the ubiquitination event is essential for the correct function of the pathways the substrate is involved in. The Protein Structure and Function lab investigates mechanisms of E3 action adopting a structural and biochemical approach.

Specificity and selectivity in the ubiquitin cascade

There are many mechanistic questions that surround the E3-substrate interaction and how specificity for both target protein and type of modification is achieved. We aim to answer these questions exploiting three model systems for the understanding of the molecular mechanisms of ubiquitination of a target molecule. One model in the lab is

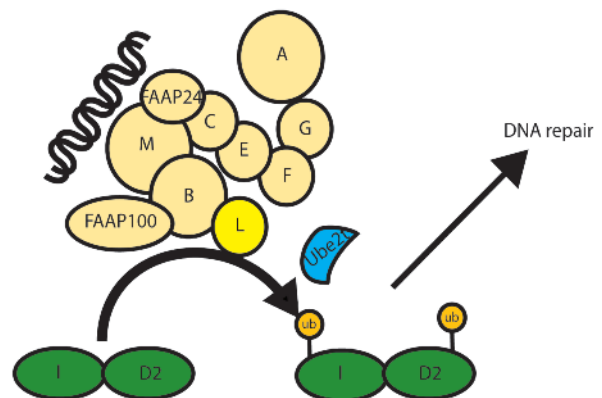


Figure 1. A cartoon representation of the mammalian Fanconi Anaemia core complex, with E2 in blue and substrates indicated in green. Ubiquitin is depicted in orange.

the Fanconi Anaemia pathway, which is a DNA repair pathway with monoubiquitination at the heart. Patients with Fanconi Anaemia are sensitive to DNA breakages, and have a high disposition to cancer. The central core of the pathway is a poorly characterised multi-subunit protein complex responsible for monoubiquitinating FANCD2 and FANCI at a specific site on each. There is no evidence of ubiquitin chain formation or other sites of ubiquitination, and therefore this pathway represents an ideal model for understanding substrate and ubiquitination specificity.

The Fanconi-Anaemia pathway

Fanconi Anaemia (FA) is a rare recessive disorder characterised by chromosomal instability and a pre-disposition to cancer. FA is divided into at least 13 complementation groups (FA-A, B, C, D1, D2, E, F, G, I, J, L, M and N). However, FA components are also implicated in other genome instability disorders such as Bloom's syndrome. FA proteins are also closely related to the BRCA pathway which is found to be mutated in many cases of breast cancer – FANCD1 is BRCA2, FANCN is PALB2 (Partner and Localiser of BRCA2), and FANCI is identical to BACH1/BRIP1, a DNA helicase that interacts directly with BRCA1. A, B, C, E, F, G, L, M and 2 uncharacterised components FAAP100 and FAAP24 form the nuclear FA core complex (Figure 1.) This complex is required for monoubiquitination of FANCD2 on lysine 561 and lysine 523 of the newly-identified FANCI, so currently there are 2 substrates for its monoubiquitination activity, but FANCI and FANCD2 share 40% sequence similarity in the region surrounding the ubiquitination site. The discovery of FANCI as a target for the complex opens up a whole new set of

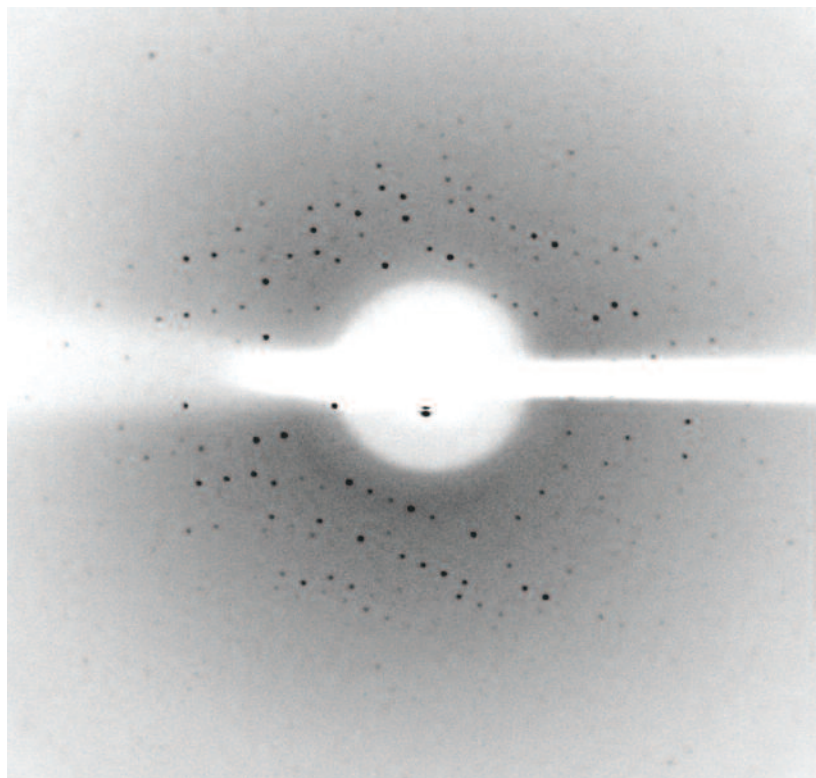


Figure 2. Typical diffraction from a crystal of a component of the FA core complex.

questions regarding the machinery, and it is still unclear what regulators of the activity of the complex might be, although Ube2T was recently predicted to be the E2 for the pathway. Of the 10 components of the core complex, only M and L have identifiable domains. FANCM has DNA helicase-like and endonuclease-like domains, suggesting it may be involved in processing DNA, and FANCL has a RING finger domain and WD40 repeats suggesting it may be the catalytic 'E3' component of the complex.

We are aiming to structurally characterise the whole core complex, in order to understand how the complex recognises and ubiquitinates FANCD2 (and FANCI), and how this event is compromised, on a molecular level, in the disease state.