Molecular mechanisms of SMG1 kinase in health and disease

Supervisory team:
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Collaborators: Prof Andreas Kulozik (University of Heidelberg, Germany), Prof Alberto Fernandez Jean (University Europea Madrid, Spain)

Host institution: University of Bristol

Project description:
Nonsense-mediated mRNA decay (NMD) recognizes and eliminates mRNAs with a premature stop codon (PTC). NMD is an important mRNA quality control mechanism and essential regulator of eukaryotic gene expression. Recognition of a PTC-containing mRNA, the event that triggers NMD, depends on translation and leads to the assembly of the dynamic NMD machinery comprising UP-Frameshift proteins UPF1, UPF2, UPF3B and the SMG1-8-9 kinase complex. UPF2 and UPF3B are suggested to stimulate UPF1 phosphorylation by SMG1-8-9, a key step in triggering deadenylation, decapping and ultimately decay of the faulty mRNA. Mutations in human genes encoding NMD factors result in severe disease; they cause neurodevelopmental disorders or specific types of tumours.

We have established protocols for production of SMG1 kinase, SMG8 and SMG9 (which inhibit SMG1 kinase by a yet unknown mechanism) and UPF proteins using multiprotein expression in mammalian cells and insect cell expression (with Berger). In addition to wild-type SMG1 we will express recently identified, uncharacterised SMG1 mutants causing intellectual disability and autism. We will investigate how NMD (mRNA stability of model mRNAs) is affected in human cells by these SMG1 mutations. Moreover, we will perform in vitro kinase assays and analyse SMG1-proteins interactions (determine affinity constants) to understand how SMG1 function is affected and thus discover new aspects of NMD regulation.

We propose to determine the structure of the SMG1-8-9 complex and its mechanisms of substrate recognition by cryo-EM. We previously studied the overall architecture of SMG1-8-9 by electron microscopy at medium resolution. However, a high-resolution structure is required to understand the molecular mechanisms of SMG1 in health and disease.

Cryo-EM, in vitro and in vivo biochemistry will synergise to reveal the underlying mechanisms of this key activation step of NMD. The project will be supported by the excellent environment and research facilities at University of Bristol (the Eukaryotic Expression Facility, the Talos Arctica cryo-microscope and the BlueCryo high-performance computing cluster).

The project offers a unique opportunity for training in state-of-the-art experimental strategies. Complementary execution of these approaches has a very high training potential and a unique breadth of laboratory skills for the prospective student, including in vivo and in vitro biochemistry, biophysics and cutting-edge molecular structure determination. First rotation will be with Berger to produce proteins in mammalian and insect cells and determine stability of reporter mRNAs (in vivo NMD), building on previously established protocols. Our medium-resolution structure of SMG1-8-9 will be used as initial model for 3D reconstruction, facilitating structure determination with Berger-Schaffitzel.

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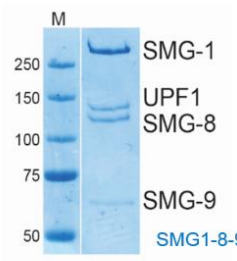
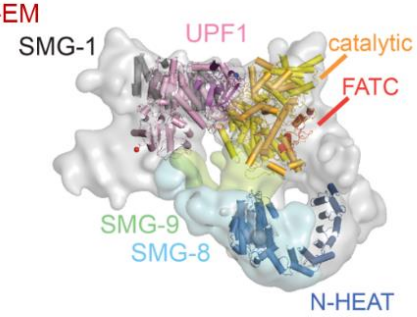
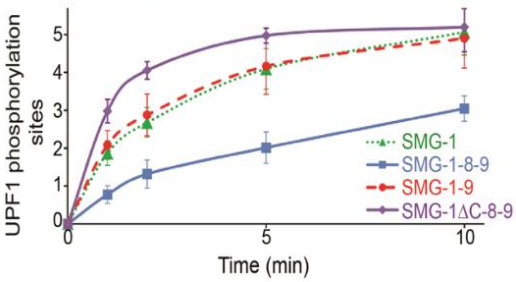
Medium resolution cryo-EM structure of SMG1-8-9

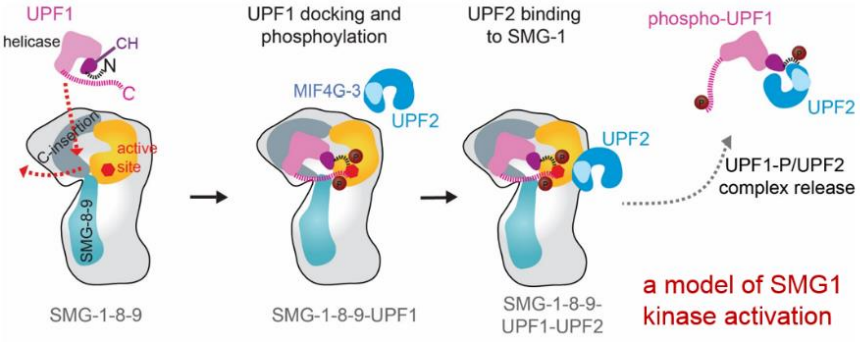


SMG1-8-9 preparation



SMG1 activity





a model of SMG1 kinase activation

SMG1 substrate recruitment