

Activation of a ubiquitin fold containing pre-mRNA splicing regulator Sde2 by deubiquitinating enzymes Ubp5 and Ubp15 in *Schizosaccharomyces pombe*

The genetic information in eukaryotes flows into protein-coding messenger RNAs through transcription and pre-mRNA splicing. A multi-megadalton protein complex, spliceosome, removes non-coding introns and joins exons to generate translatable mRNAs. It is made of five small nuclear ribonucleoproteins (snRNPs) and more than 200 proteins. In addition, many protein/RNA interactions and post-translational modifications play key roles in regulating the machinery. Ubiquitin and ubiquitin-like proteins (UBLs) are post-translational modifiers functioning in protein degradation, cell cycle, transcription, immune defence, as well as in splicing regulation. Ubiquitin or UBLs share a β -grasp fold and are synthesized as precursor molecules which get processed and activated by deubiquitinating enzymes (DUBs) or UBL-specific proteases respectively. These enzymes are highly substrate specific and are essential for ubiquitin and UBL associated pathways.

In my thesis work, we report the mechanism of activation of a novel ubiquitin-like protein Sde2 by deubiquitinating enzymes (DUB) Ubp5 and Ubp15. Sde2 is an intron-specific pre-mRNA splicing factor required for splicing of selective pre-mRNAs in *Schizosaccharomyces pombe*. The structure of Sde2 has an N-terminal ubiquitin-like fold (Sde2-UBL) followed by a conserved GG~KGG motif and a C-terminal helical domain (Sde2-C). The precursor gets processed at the GG~K site by Ubp5 and Ubp15. Processing of Sde2 precursor is required for its association with the spliceosome; thereby, processing defective mutants of Sde2 show $\Delta sde2$ -like splicing defects. The Sde2-UBL is essential for generating the functional Sde2-C with a lysine at its N-terminus. Chromosomal mutations of this lysine resulted in reduced recruitment of another splicing factor Cay1 in the spliceosome and also showed splicing defects.

Strikingly, *S. pombe* Ubp5 and Ubp15 cleave both Sde2-UBL and ubiquitin which are less than 20% identical to each other. However, a homolog of these DUBs in humans, USP7, processes ubiquitin but not Sde2. By swapping the domains of USP7 with Ubp15, we have narrowed down a region in Ubp15 that is responsible for its dual specificity towards Sde2-UBL and ubiquitin. Thus, we have demonstrated that two DUB paralogs activate distinct UBLs with roles in diverse processes related to the ubiquitin system and pre-mRNA splicing. Finally, I will also discuss how these two processes might be connected in the biological system.