Role of the ubiquitin-related protein Sde2 in intron-specific pre-mRNA splicing

SYNOPSIS

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Introduction

The cellular form of life is mediated by the transfer of genetic information from genes to messenger RNAs (mRNAs) that encode for proteins. The protein-coding genes in eukaryotes – often interrupted by non-coding introns – are transcribed as precursor mRNAs (pre-mRNAs). Therefore removal of the introns and generation of mRNAs through the process of pre-mRNA splicing becomes a prerequisite for protein translation. The excision of introns and joining of exons is facilitated by a large ribonucleoprotein (RNP) complex called spliceosome. Introns vary in length, position in the pre-mRNA, secondary structures, splicing signals (5' splice site, branch point, 3' splice site), and distances between the signals. These variations become crucial for gene expression as well as to promote alternative splicing, whereby a gene gives rise to more than one mRNA. Here regulators like RNA binding proteins (RBP), RNP modifying enzymes (ATPases, helicases), and ubiquitin-like modifiers (UBLs) become important for proper splicing.

UBLs are often synthesized as precursors that get processed after a conserved di-glycine motif by respective UBL-specific proteases. UBLs are key regulators of many cellular processes including protein degradation, DNA repair, transcription, and ribosome biogenesis. UBL-mediated modifications of spliceosomes have also been reported to regulate pre-mRNA splicing. For example, the UBL Hub1 regulates alternative splicing of *SRC1/HEH1* in the budding yeast *Saccharomyces cerevisiae* by promoting the usage of non-canonical 5' splice sites. Hub1 modifies spliceosomes by binding non-covalently with HIND-containing splicing factors Snu66 and/or Prp38 and activates the RNA helicase Prp5 for its function[1,2]. However, how spliceosomes ensure precise excision of diverse introns in a time, kinetic and expression-specific manner in intron-rich organisms is not well understood.

Objectives of the study

In contrast to *S. cerevisiae*, wherein the deletion of Hub1 results in only mild growth defects, the protein becomes essential for viability in *Schizosaccharomyces pombe.* This difference in phenotypes in the two yeasts could be attributed to higher number of

introns and alternative splicing events in the intron-rich fission yeast. To search for more Hub1-like regulators of spliceosomes, we performed a genetic screen with a temperature sensitive mutant of *hub1* in *S. pombe*. Consistent with the role of *hub1* in pre-mRNA splicing, several splicing factors showed synthetic sickness with *hub1* mutant in addition to a ***sde2*** deletion strain. A predicted structure of Sde2 showed the presence of a N-terminal ubiquitin-fold with a conserved di-glycine (GG) motif followed by a C-terminal domain (henceforth referred to as $Sde2_{UBL}$ and Sde2-C, respectively). The main objective of my doctoral study was to understand the function and mechanism of the ubiquitin-fold protein Sde2.

Sde2 was reported to be present in spliceosomal purifications[3,4], and was shown to regulate telomeric silencing and genome stability[5]. The centromeric desilencing in *sde2* deletion strain was linked to defects in processing of centromeric outer repeats and cytoskeleton constituents[4]. Recently, PCNA-dependent cleavage and degradation of human Sde2 protein was reported to regulate UV-induced replication stress[6].

Outcome of the study

Sde2 is processed like ubiquitin precursors

In my study, I demonstrate that Sde2 is a new member of the UBL family. Sde2 protein is conserved from fission yeast to humans, but *Saccharomyces cerevisiae*, *Candida albicans,* and *Pichia pastoris* lack an obvious homolog of the protein. It is synthesized as a precursor with N-terminal $Sde2_{UBL}$ flanked by an invariant di-glycine containing motif GG~KGG and a C-terminal Sde2-C domain. We show that $Sde2_{UBL}$ gets cleaved after the first di-glycine motif to generate Sde2-C starting with a lysine. The cleavage of the precursor is essential for the function. Interestingly, $Sde2_{UBL}$ was replaceable with processing efficient UBLs, ubiquitin and Ned8. Sde2 is indeed a *bona fide* UBL was confirmed by the fact that the precursor gets cleaved at the di-glycine motif like other UBL precursors, and cleavable ubiquitin and Ned8 could functionally replace the ubiquitin fold of Sde2.

Processed Sde2-C is an integral component of the spliceosome

Deletion of *sde2* gene in *S. pombe* shows sensitivity to various stresses like high/low temperature, bleomycin, thiabendazole, valproic acid and sodium butyrate[5]. Complementation assays in *sde2* deletion strain with isolated domain elucidated that Sde2-C is the functional domain. Following processing, Sde2-C associates with core Prp19 complex subunits of the spliceosome, Prp19, Isy1 and Cdc5.

Sde2 has a role in intron-specific pre-mRNA splicing

To study the role of Sde2 in pre-mRNA splicing, we monitored intron-containing transcripts in *sde2* mutants using splicing-sensitive microarrays designed for *S. pombe*. We found that Sde2 is a unique regulator of the spliceosome as it is required for splicing of only a subset of genes. Intriguingly, it functions as a intron-specific splicing factor and promotes efficient excision of only selected introns from its target pre-mRNAs.

Sde2 is required for telomeric silencing and genomic stability

The majority of splicing targets of Sde2 have functions in cellular processes such as transcription, replication, chromatin silencing, and chromosome segregation. The processing of Sde2 is also required for intron-specific pre-mRNA splicing. The protein appears to be a critical splicing regulator for proper expression of selected factors functioning at the chromatin. Absence of Sde2 or defects in its processing both resulted in aberrant telomeric silencing and defects in chromosome segregation.

Sde2 facilitates association of Cay1 with the spliceosome

We found that Sde2 mediates recruitment of Cactin/Cay1 to the spliceosome (Cay1 was reported to regulate pre-mRNA splicing in *S. pombe*[7]). We showed that Cay1 also functions as an intron-specific splicing factor like Sde2, as *cay1* and *sde2* mutants interacted genetically and showed intron-specific pre-mRNA splicing defects for same pre-mRNA targets. Thus Sde2 functions together with Cactin to promote precise excision of specific introns from selected genes.

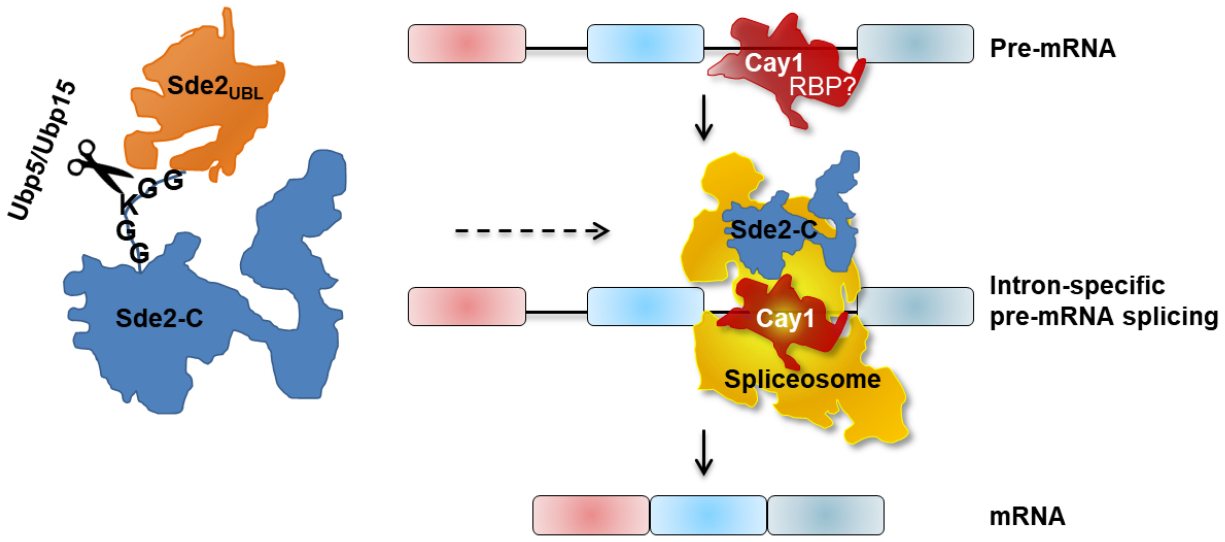

Figure: Schematic of Sde2's function in intron-specific pre-mRNA splicing.
Sde2 precursor (shown at the left) is comprised of a ubiquitin-fold ($Sde2_{UBL}$) and Sde2-C domain connected with highly conserved GGKGG motif. After processing, Sde2-C starting with the lysine becomes part of the spliceosome and promotes excision of selected introns from a subset of pre-mRNAs. In a parallel study by a colleague Prashant A. Pandit, two deubiquitinating enzymes, Ubp5 and Ubp15, were shown to cleave Sde2 after the first GG motif. Sde2-C facilitates association of Cactin/Cay1 and possibly other RNA binding proteins (RBP) with the spliceosome.

Conclusion

Through my doctoral study I have established a direct link between ubiquitin-like processing and the process of pre-mRNA splicing by the spliceosome. I have discovered a ubiquitin-fold-containing splicing factor Sde2 which regulates splicing of a subset of genes in an intron-specific manner. Key targets of this regulator have critical functions in heterochromatin silencing and chromosome segregation. Thus, I propose that Sde2-dependent intron-specific pre-mRNA splicing might function as a checkpoint to ensure telomere homeostasis and genome stability.

The processing of $Sde2_{UBL}$ to generate spliceosomal Sde2-C bears intriguing resemblance to the processing of the ubiquitin-ribosomal fusions to produce ribosomal proteins[8]. Thus the two major RNP machineries of the cell, the ribosomes and spliceosomes, appear to share a common principle of regulation via ubiquitin-like processing.

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