Talk

Functional characterization of splicing factor Cwf15 in the maintenance of genomic stability

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ABSTRACT

Motivation: The accumulation of mutations and rearrangements, or the asymmetrical distribution of genetic material during the mitotic process lead to cellular defects known generically as genomic instability. Previously, we isolated a truncated version of cwf15 lacking it last 53 aa (cwf15.d53) which presents two clearly deleterious phenotypes associated with genomic instability: readthrough and aberrant distribution of the cohesin loaded in the chromosomes. These data show that, in addition to its function in splicing, cwf15 plays a fundamental role in the end of transcription and in the charge/discharge cycle of cohesin. The specific objectives of this project are: Expression in trans of the 53 amino acid that lacks the mutant to evaluate self-contained function. Testing in vivo a “proof of concept” design to follow specifically the loaded cohesin in the chromosomes. Finding extragenic suppressors of cwf15 defective function.

Methods: First, we characterized the chromatin segregation defects of cwf15 and compared quantitative-and-quantitatively to other splicing mutant to show that there exist functional differences. Second, we have engineered, and inserted into the genome, different control and experimental expression constructs to assess functional complementation of Cwf15 c-terminal domain in trans. Third, we have designed and constructed a novel system by bi-molecular complementation to monitor cohesin specifically loaded onto the chromosomes in living cells. Fourth, we have generated genetic combinations of cwf15 deletion and truncation with very recently published mutations in transcription termination genes (yth1 and seb1) to show that cwf15 is involved in a RNA processing different from splicing.

Results and conclusions: We show that defective cwf15 function phenotype is different from other characterized splicing factors and that a specific gain-of-function mutation in transcription termination seb1 is able to suppress growth defects of cwf15.d53 mutant. Furthermore, a missense mutation in the RNA polII pausing factor yth1 also rescues lethality of cwf15 whole deletion. On the other hand, preliminary experiments suggest that expression in trans of the last 53 amino acids does not reverse the phenotype of cwf15.d53 although it accumulates in the nucleolus.

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