mRNAs Encoding Zinc Finger Protein Isoforms are Expressed by Alternative Splicing of an In-frame Intron in Fission Yeast

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Abstract

We report here that a gene encoding a protein with three zinc fingers is expressed predominantly to produce a protein containing only two zinc fingers in the fission yeast Schizosaccharomyces pombe. A third zinc finger resides within the in-frame intron that is normally spliced out. By RT-PCR analysis, we detected a minor transcript encoding a protein with three zinc fingers. Such alternative splicing for assortment of zinc finger domains have been reported in animals and implicated in switching of the target genes expressed specifically during development. This is the first report of the occurrence of such zinc finger assortment in lower eucaryotes.

Key words: Schizosaccharomyces pombe; zinc finger; alternative splicing

Zinc finger domains bind DNA and/or RNA by recognizing specific nucleotide sequences. Some genes encoding several distinct zinc fingers are alternatively spliced and produce proteins with different combinations of zinc finger domains resulting in recognition of different targets. Such assortment of zinc fingers by alternative splicing has been reported in animals, and implicated in switching of the transactivation of different target genes under different developmental stages. Here we report that such zinc finger assortment by alternative splicing presumably occurs in the fission yeast Schizosaccharomyces pombe.

We isolated a mutant (Ts34) showing temperature-sensitive growth with a defect in nuclear division at the restrictive temperature of 34°C (to be published elsewhere). By searching for a clone to complement this mutant, we identified a gene and its corresponding cDNA. Comparing the sequences of the genomic DNA and the cDNA, we found that the gene contained nine introns (Fig. 1A, B). The deduced amino acid sequence of the cDNA showed that this gene encodes two C2H2 zinc finger motifs in the first and second exons (Fig. 1C). The reading frame of the second exon continues through the second intron into the third exon. The deduced amino acid sequence of the second intron revealed the presence of another C2H2 zinc finger motif (Fig. 1C). To know whether the third zinc finger embedded within the read-through sequence containing the in-frame intron is expressed or not, we analyzed the mRNA of this gene by reverse-transcription polymerase chain reaction (RT-PCR). With primers that anneal respectively within the first exon and the fifth exon (Fig. 1A), the reverse transcript of the poly(A)+ RNA from exponentially growing haploid S. pombe was amplified and cloned. Of the 65 clones randomly picked up and sequenced, 62 contained the sequence of fully spliced mRNAs and one unspliced mRNA. The remaining two contained an mRNA sequence that contained the second intron. Thus the mRNA capable of encoding the third zinc finger as well as that of the first and second zinc fingers were indeed expressed in S. pombe cells. We named the gene zasl+ (zinc fingers alternatively spliced), the protein with two zinc fingers ZaslA, and the protein with three zinc fingers ZaslB (Fig. 1B).

The Ts34 mutation was identified in the zasl gene (Fig. 1A). Therefore, this mutation (hereafter zasl-34) is presumably responsible for the temperature-sensitive phenotype. Because the cDNA encoding ZaslA complemented the temperature-sensitive growth defect of the mutant, we think that the third zinc finger encoded by ZaslB is not necessary for mitotic growth. Ts34 also showed sporulation defects even at the permissive temperature for growth of 26°C. It would be interesting to know the functions of ZaslA and ZaslB during sexual development. However we do not have data as to whether ZaslB is expressed as a functional protein or not.

ZaslA showed weak overall homology with AmdA (accession number S61908, 22% identical and 36% similar amino acids within 629 residues) of Aspergillus nida-
Saccharomyces cerevisiae within 226 residues) of cal and 60% similar amino acids within 70 residues) and between ZaslA and other zinc finger proteins, such as or YPR022c (38% identical and 57% similar amino acids within 70 residues) is not significantly higher than those between ZaslA and other zinc finger proteins, such as Adr1 (P07248, 47% identical and 64% similar amino acids within 57 residues), YMI1 (Q04545, 45% identical and 60% similar amino acids within 70 residues) and ZMS1 (P46974, 44% identical and 62% similar amino acids within 70 residues) of S. cerevisiae, and human SPI (P08047, 44% identical and 61% similar amino acids within 63 residues), WT1 (P19544, 39% identical and 55% similar amino acids within 74 residues) and EGR-3 (Q06889, 41% identical and 52% similar amino acids within 77 residues). Zinc fingers sharing strong homology with the third zinc finger of ZaslB were not found.

Regulated splicing under specific conditions has been reported with mesl+ of S. pombe\textsuperscript{5} and MER2 of S. cerevisiae\textsuperscript{10,12} during meiosis, and with HAC1 of S. cerevisiae responding to unfolded protein.\textsuperscript{11} Molecular mechanisms of the specific splicing have been genetically investigated.\textsuperscript{10-13} zasl+ of S. pombe is the first instance of alternative splicing in yeasts. It may provide an example suitable for investigating the mechanisms of alternative splicing.

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