

### 32. A new repetitive sequence, *Mitugo*, found in the 5'-terminal regions of rice genes

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Rice has three *SnRK1* (*sucrose non-fermenting-1 related protein kinase 1*) genes, that are classified into two sub-families, *SnRK1a* (*OSK1*) and *SnRK1b* (*OSK24* and *OSK35*) on the basis of amino acid sequence similarities and expression patterns (Takano et al., 1998, Kanegae et al., 2005). The three genes consist of eleven exons and ten introns, with the initiation codons residing in the second exons, and the stop codons in the eleventh exons, respectively. The nucleotide sequences of *OSK24* and *OSK35* are highly conserved from the middle of the first intron to the eleventh exon regions (90% identical). In contrast, they show no homology to each of the other regions from the first exon to the middle of the first intron (Fig. 1). Interestingly, the genomic Southern hybridizations showed that the first exon of *OSK24* contained a highly repetitive sequence, while that of *OSK35* was composed of a unique sequence (data not shown).

We used BLAST program (Altschul et al., 1990) against DDBJ/GenBank/EMBL DNA databases (<http://www.ddbj.nig.ac.jp>) to find repetitive sequences in the 5'-terminal regions and the upstream regions of these two genes. A novel interspersed repetitive sequence of 452 bp in length, as well as several known transposons, was found in *OSK24*, but not in *OSK35* (Fig. 1). The novel repetitive sequence consisted of a 10-bp upstream sequence, the first exon and the non-homologous region of the first intron of *OSK24* (Fig. 1). We named it *Mitugo* ("triplets" in Japanese) because characteristic three tandem repeats were contained, whose unit lengths were about 110 bp (Fig. 2A). However, there were no characteristics of known transposable elements or repetitive sequences, such as terminal inverted repeats.

By a homology search using the *Mitugo* sequence in *OSK24* (designated *Mitugo1*) as a query, 62 additional *Mitugo* members were identified in the *O. sativa* Nipponbare genome. A BLAST score lower than  $e^{-5}$  was taken into account. These *Mitugo* members were dispersed on every chromosome. The number of tandem repeats in their sequences varied from 0 to 3 (see Fig. 2B, showing that *Mitugo3* and *Mitugo7* have two repeats). In about two thirds of the members, such as *Mitugo3* and *Mitugo7* in Figure 2B, the sequence homology extended to the upstream region, unlike in *Mitugo1*. This suggests that *Mitugo1* is a truncated member.

It has been reported that the 5'UTR of rice mitochondrial protein S10 genes (*rps10*) had homology to *OSK24* and other rice transcripts (Kubo et al., 2000). We found that the 5'UTR of *rps10* genes have *Mitugo* sequences shorter than *Mitugo1*, which suggests that they also have truncated *Mitugo* members (data not shown). A detailed analysis of the *Mitugo* member sequences in the rice genome will be published in another paper.

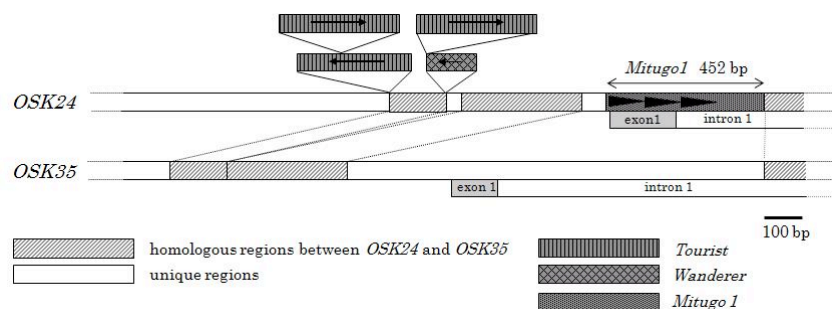


Fig. 1 Promoter regions of *OSK24* and *OSK35*. *Mitugo1* and transposable elements (*Tourist* and *Wanderer*) are represented by boxes with different patterns. Triangles indicate tandem repeats in *Mitugo1* and arrows show directions of transposable elements. Hatched boxes indicate homologous regions between *OSK24* and *OSK35*, and open boxes are unique regions. Positions of first exons and first introns are underlined. Bar represents 100 bp.

To evaluate how often the *Mitugo* members are found in genic regions, homology searches were performed using each *Mitugo* and its flanking sequences as the query in full-length rice cDNA clones (KOME; <http://cdna01.dna.affrc.go.jp/cDNA/>; Kikuchi et al., 2003) and RAP-DB (RAP; <http://rapdb.dna.affrc.go.jp/>; Rice Annotation Project, et al., 2008). Seventy percents of the *Mitugo* members exist in independent annotated genes in the same direction as *Mitugo1*. More than 90% of them overlap with

