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Retron Integration as a Replacement of Pre-existing DNA Fragment

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Retron is a bacterial genetic element with reverse transcriptase. In general, retrons in natural E. coli isolates are different from each other in their nucleotide sequence. It was suggested that different retrons were independently integrated into E. coli chromosome, but it is not known the source of retron nor the mechanism of integration. To understand the mechanism of retron integration, we have studied E. coli chromosomal sites integrated by retron EC86, the retron isolated from E. coli B. In E. coli B the retron EC86 is integrated into the defective prophage. Comparison of the defective prophage before and after retron integration shows that the retron EC86 replaces about 3.5 kb phage DNA containing two open reading frames. We suggest the replacement of pre-existing DNA fragment as a retron integration mechanism. Supported by KOSEF 931-0500-008-2p

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Construction and Expression of N-Terminal Domain Swapped Citrate Synthase Genes in Saccharomyces cerevisiae

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Saccharomyces cerevisiae contains two distinct nuclear genes, CIT1 and CIT2, encoding mitochondrial (CS1) and nonmitochondrial (CS2) citrate synthase, respectively. As a part of an effort to understand the mechanism of intracellular organelle targeting of the citrate synthases, we have determined the N-terminal sequences of CS1 and CS2. The N-terminal sequences of CS1 was determined to be S-S-A-S-E-Q-T, which indicates that 7 N-terminal amino acids of the precursor CIT1 protein are removed by the cleavage as R(35)-H-Y ↓S(38) in the process of mitochondrial targeting. The N-terminal amino acid sequence of CS2 was revealed to be L-Q-S-N-S-S, which suggests that the 15 N-terminal amino acids are cut off from the precursor CIT2 protein by the cleavage as A(13)-S-Y↓L(16) during its import into peroxisomes in spite of the presence of C-terminal peroxisomal targeting signal (PTS1), S-K-L.

In order to understand the roles of the N-terminal sequence of precursor CIT2 protein in the process of targeting into peroxisomes, the N-terminal parts of the CIT1 and CIT2 genes were exchanged reciprocally to yield two chimeric genes, CIT12 and CIT21. The chimeric genes were found to complement the glutamate auxotrophy of S. cerevisiae SP1 lacking both citrate synthases activity. By immunoblot analysis, it was also revealed that both the hybrid genes are properly expressed in S. cerevisiae SP1. Nevertheless the cell lysate of SP1/CIT12 transformant showed no detectable citrate synthase activity in the transformants, while that of SP1/CIT2 transformant showed very high activity.