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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 37 *N*-terminal amino acids of the precusor 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 precusor 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 precusor 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 activitiy. 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/*CIT12* transformant showed very high activity.