S4

Transposable Elements Arrangement in Genome and Their Applications for Analysis of Evolutional Events

Hideaki Maekawa

Division of Radiological Protection and Biology, National Institute of Infectious Diseases

Abstract

The ribosomal RNA gene (rDNA) cluster was located in the nucleolus organizer and was genetically determined as one locus. We speculated by using sequence differences in the functional rDNA unit that the segregation time between Chinese and Japanese types of *B. mandarina* is about three million years ago. The differences of the amount of inserted non-LTR retrotransposons, R1Bm and R2Bm, in rDNA cluster were used for the identification of *B.mori* strains. The analysis of isolated old type element carrying tripartite structure comprising a *mariner*-like element and two additional retrotransposons, BMC1 and L1Bm, could demonstrate the relationships between LINE (Long INterspersed Element) and SINE (Short INterspersed Element). Another DNA mediated type of transposable element, transposon *mariner*, was recognized as a very useful tool for analysis of evolutional event in Lepidoptera and horizontal transfer into several species inhabiting in geologically close area.

Introduction

A lot of transposable elements are contained in genomic DNA of almost eukaryote. Over 30% of the genome in almost eukaryotes might be occupied by those elements. Two major elements, retrotransposon and transposon, are well known. These are an RNA mediated type and a DNA mediated type, respectively. L1 or LINE1 is the major group of retrotransposons and codes a kind of polymerase including endonuclease and reverse transcriptase domains. These elements are considered that the own RNA transcripts recognized by the translated enzyme complex are integrated into the genome by target primed reverse transcription (TPRT)(Luan and Eickbush, 1995). Another group, transposon for example *mariner* (Nakajima et al., 1998), codes only the transposase and the enzyme has activities of excision and insertion from DNA by the recognition of inverted repeats flanking with both ends of the element and into the target sequence such as TA, respectively.

Non-LTR retrotransposons, R1Bm and R2Bm (Fujiwara et al., 1984), are mainly located on the ribosomal RNA gene (rDNA) cluster which constructs one locus (Maekawa *et al.*, 1988;Tsuchida *et al.*, *1995) with tandem repeating array of about 240 copies of the 10.6kb functional unit in silkworm, Bombyx mori.* These retrotransposons are not eliminated from the cluster and the sequences are conserved, and therefore, these comparative contents (those insertion amount depending on the strain is variable up to 60%.) in the cluster were able to use for identification of each strain of *B.mori.* The group of R1 and R2 that is classified for retrotransposons located in rDNA has been usually found in insects. These insertion events might be occurred at the long time ago, at least before separation of Lepidoptera and Diptera.

However, the function of these elements is not known yet. This unknown situation has been similar for the other L1 elements including the BMC1 element (about 0.2% of *B. mori* genome) that is found as dispersed distribution only in the *B.mori* genome (Ogura et al., 1994).

These elements are useful tools for analysis of evolutional events and identification of strains (Ninaki et al., 1989;Funatsuki et al., 2001) in insects. The separation time between *B.mandarina* (China) and *B. mandarina* (Japan) was roughly calculated by using the pattern analysis of rDNA cluster (Maekawa et al., 1988). The tripartite structure comprising a *mariner*-like element and two additional retrotransposons was conserved in almost *B.mori* strains (Nakajima et al., 1999). The evolutional story of BMC1 and L1Bm were speculated by analysis of these sequences. The *mariner*-like elements were also used for determination of evolutional relationships among Lepidopteran species (Nakajima et al., 1998;Nakajima et al., 2002).

We want to talk whether these transposable elements are necessary for maintenance of genome, or not and to discuss as a possible useful vector for introduction of genes (Okano et al., 1998).

Materials and Methods

1. The genomic DNAs were extracted from silk glands of silkworm, *B. mori* strains and *B. mandarina*.
2. Southern blot hybridization was carried out for analysis of rDNA cluster. In the rDNA cluster, three major type units were included which there are a functional and non-insertion type of 10.6kb, an insertion type with R1Bm of 15.7kb and another insertion type with R2Bm of 15.1kb. The rDNA probes containing the 10.6kb unit was used for hybridization.
3. PCR amplification and sequencing were done by using apparatuses of MJ Research and Parkin Elmer(ABI), and ABI and Li-Cor, respectively.
4. Multi-alignment and phylogenic tree were calculated by the GCG program and the ClustalX software.
5. The FISH method was used for visual analysis of distributions on chromosomes of rDNA and BMC1.

Results and Discussion

1. We found different functional unit of rDNA in *B. mandarina* living in Japan. At least two restriction enzyme sites (*Eco*RI and *Kpn*I) were different between the spacer regions of two functional units of *B.mori* and *B. mandarina*. Some 28S rRNA genes of *B.mori* were inserted with retrotransposon, R1Bm or R2Bm and then a functional 10.6kb unit changed to increased size of 15.7kb and 15.1kb, restrictively. We could easily distinct *B.mandarina* living in Japan from *B.mori* by comparison of whole structure of rDNA. Therefore, we calculated three million years as time enough that one novel unit of the rDNA cluster which means a mutation in a multigene family, would be fixed in the whole region of the cluster by uncrossing over. This three million years was roughly coincident with about two million years ago when the geological separation occurred corresponds to the middle of the Pleistocene when the Korean Peninsula was formed by the subsidence of Hwang Hai (Yellow Sea). (Fujiwara et al., 1984;Maekawa et al., 1988;Luan and Eickbush, 1995;Tsuchida et al., 1995)
2. In *B. mori*, amounts of retrotransposons, R1Bm and R2Bm, inserted into rDNA were variable and

depended on strains. Southern blot hybridization could detect different amount of retrtransposons among the strains for the identification. (Maekawa et al., 1988;Funatsuki et al., 2001)

3. The BMC1 was major non-LTR retrotransposon in the *B.mori* genome and dispersed on all chromosomes. And BMC1 was specific to *B. mori* and target duplication occurred in the insertion site. The same family member designated as L1Bm was found. Both members have the highly conserved sequence in the 3' UTR which we propose a recognition site for retrotransposition mechanism conducted by a reverse transcriptase and an endonuclease complex. The PCR amplified clone using inverted terminal repeats of *mariner*-like sequence as a primer, had a size of about 4.2kb and, after sequencing, was found to contain an L1Bm, which was in turn integrated with BMC1. This novel tripartite structure was found in almost *B.mori* strains but not in *B. mandarina* (Japan). (Ogura et al., 1994;Nakajima et al., 1999)

4. The DNA mediated transposon, *mariner* including *mariner*-like elements (MLEs), is found in many species from plant to human. The ORF (open reading frame) of transposase was detected in only few species including *Drosophila mauritiana* and Emperor moth, *Attacus atlas*, but not in *B. mori*. The consensus sequence in the transposase is a useful tool for phylogenic analysis. Some invertebrates inhabiting in the close area have highly conserved full-length MLEs. The analysis of this full-length unit isolated by use of inverted terminal repeats also showed the geological relationships among species containing MLEs. (Nakajima et al., 1998;Nakajima et al., 2002)

References

1. H. Fujiwara et al., 1984, Introns and their flanking sequences of *Bombyx mori* rDNA. Nucleic Acid Res. 12, 6861-6869.
2. K. Funatsuki et al., 2001, Rapid Identification of *Bombyx mori* cells using PCR amplification following a direct procedure for genomic DNA preparation. J. Insect Biotech. Sericol. 70, 129-136.
3. D.D. Luan and T.H. Eickbush, 1995, RNA template requirements target DNA-primed reverse transcription by the R2 retrotransposable element. Mol. Cell. Biol. 15, 3882-3891.
4. H. Maekawa et al., 1988, Nucleolus organizers in the wild silkworm *Bombyx mandarina* and the domesticated silkworm *B. mori*. Chromosoma 96, 263-269.
5. Y. Nakajima et al., 1998, Isolation of a *mariner*-like sequence containing a complete open reading frame for transposase from *Attacus atlas* and its phylogenetic relationships within the Ditrysia of Lepidoptera. J. Seric. Sci. Jpn. 67, 271-278.
6. Y. Nakajima et al., 1999, A novel tripartite strucutre comprising a *mariner*-like element and two additional retrotransposons found in the *Bombyx mori* genome. J. Mol. Evol. 48, 577-585.
7. Y. Nakajima et al., 2002, Possible horizontal transfer of mariner-like sequences into some invertebrates including Lepidopteran insects, a grasshopper and a coral. J. Insect Biotech. Sericol. 71, 109-121.
8. O. Ninaki et al., 1989, Gene analysis by blot hybridization on the silkworm, *Bombyx mori*, cell lines. In Invertebrate Cell System Applications, CRC Press, vol.1, 143-149.

9. T. Ogura et al., 1994, A defective non-LTR retrotransposon is dispersed throughout the genome of the silkworm, *Bombyx mori*. Chromosoma 103, 311-323.
10. K. Okano et al., 1998, A defective type of non-LTR retrotransposon, BMC1, is a useful candidate as a vector for gene introduction into cultured cells of silkworm, *Bombyx mori*. Appl. Entomol. Zool. 33, 247-254.
11. K. Tsuchida et al., 1995, Determining gene location on chromosomes of *Bombyx mori* using fluorescence *in situ* hybridization (FISH). Appl. Entomol. Zool. 30, 225-230.