SY-2 Approaches of chromosome analyses for the eastern North American *Trillium*

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In eastern North America, *Trillium grandiflorum* grows around the Great Lakes area, and *T. erectum* is distributed throughout the Appalachian mountain region. This report provides chromosome data on karyotypes derived from cold-induced heterochromatin banding of the chromosomes on materials that have been collected from each locality within each species.

1) Chromosome Composition in Populations

T. grandiflorum shows a fairly homogeneous composition within and among populations. In contrast, the chromosome structure of *T. erectum* shows fairly heterogeneous within populations. Why did such a different chromosome composition between species come about? It is found that this differences in chromosome structure is related to the evolutionary process and the fertilization mechanism in each species. No hybrids have been found in flowering areas where *T. grandiflorum* and *T. erectum* overlap. Both species have developed self-fertilization.

2) Occurrence of Supernumerary Chromosomes

Five populations out of 16 *T. erectum*, and four populations out of 25 *T. grandiflorum* were found to posses supernumerary chromosomes. From an analysis of *T. grandiflorum* the supernumerary chromosomes occurred by chromosome fragmentation. It is considered that the occurrence of supernumerary chromosome is related the evolutionary trend of the species. Asian *Trillium* species have no supernumerary chromosomes, but have developed the polyploid system through hybridization in speciation.

3) References

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SY-3 Evolution and function of heterochromatin (RCRO) in apes

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Humans and chimpanzees share very similar cytogenetic karyotypes. Significant chromosomal structural differences include a number of peri- and paracentric inversions, and the fusion of chimpanzee chromosomes 12 and 13 to form human chromosome 2. Additionally, more than half the 48 chimpanzee chromosome arms have large terminal blocks of constitutive heterochromatin (C-bands) (Marks 1985; Yunis et al. 1980), subterminal satellite DNA (Royle et al. 1994), and subterminal and interstitial telomeric sequence arrays (Hirai 2001), which are absent from humans. On the other hand, a human-chimpanzee comparative genome analysis revealed that the difference between the chimpanzee and human genomes at the nucleotide level was 1.23% (Fujiyama et al. 2002). In fact, however, the phenotypes of these two species are distinct from each other. What, besides gene differences, makes us human? Are any of the phenotypic differences between humans and chimpanzees due to chromosomal differences between these two species? These questions are not easily answered, but need to be investigated in the post-genome era using new insights.

The human genome includes 45% repetitive arrays derived from transposable elements. Segmental duplications are also a notable feature of the human genome (International Human Genome Sequencing Consortium 2001). The human genome sequencing project suggested that it might be intriguing to investigate whether

such genomic upheavals caused by repetitive sequences and duplications have a role in speciation events. This talk presents some qualitative evidence that the genomic "wasteland" seems to influence chromosome configuration and pairing and chiasma formation in male meiosis of chimpanzees, and postulates that it may bring about gene silencing and a bias in gene shuffling.

I dissected the terminal C-bands specific for chimpanzee chromosomes using a molecular cytogenetic technique, PRINS, with primers for telomeric sequences, subterminal satellite, and retrotransposable elements (HERV-K and -W). These DNA elements jointly formed a large block of retrotransposable compound repeat DNA organization (RCRO) at the terminal C-band regions of 30 chromosomes, and are also located at the centromeric regions of some chromosomes. Additionally, a block consisting of all members of the RCRO has transposed to the middle (q31.1) of the long arm of chromosome 6, and three members, the subterminal satellite and the two HERVs, have integrated into the proximal region (q14.4) of the long arm of chromosome 14. Terminal RCROs seem to induce and prolong the bouquet stage in meiotic prophase, and to affect chiasma formation, together with interstitial RCROs. It is also postulated that RCROs may cause a position effect to gene expression, resulting in gene silencing and/or late replication. Finally, I would like to show a preliminary data of the phylogenetic feature of RCRO in primates.

SY-4 An HP1 homolog, TFL2 represses flowering genes but does not affect heterochromatin in Arabidopsis

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TERMINAL FLOWER 2 (*TFL2*) gene, which is also called *LHP1* (*LIKE HP1*), encodes a homolog of Heterochromatin Protein 1 (HP1) of the animals and Swi6p of fission yeast. *TFL2* is a unique gene in the Arabidopsis genome.

tfl2 mutant shows pleiotropic phenotypes. One of the most characteristic phenotype among them is the early flowering in a day-length independent manner. Genetic and gene expression analyses revealed that this early flowering phenotype is due to the up-regulation of a flowering pathway integrator gene, *FLOWERING LOCUS T* (*FT*). *FT* gene lies downstream of long-day signaling pathway.

The other target of *TFL2* in the flowering pathway is *FLOWERING LOCUS C* (*FLC*). *FLC* integrates vernalization and autonomous pathway and represses *FT* and *SOC1* genes. Plants remember the cold treatment (vernalization) to promote flowering, but this memory will not inherit to the next generation, so that vernalization response is the epigenetic phenomenon. After the vernalization, *FLC* expression level is maintained to be low so that flowering is promoted. In the *tfl2* mutant, however, *FLC* expression restored to the untreated level promptly. We also found that TFL2 binds to the first intron of the *FLC* gene by using the chromatin immuno-precipitation (ChIP) experiment.

Since the pleiotropic phenotypes of *tfl2* mutant suggest that *TFL2* has multiple target genes other than *FT* and *FLC*, we performed expression analysis using microarray. The results suggested that several floral homeotic genes and Late embryonic abundant (*LEA*) genes are the targets of TFL2, but these genes are exclusively up-regulated and adjacent genes are nor affected by the *tfl2* mutation. Heterochromatic genes and transposons, which are expressed in the low DNA methylated mutants, were not expressed in the *tfl2* mutant. Cytological observations show that TFL2 protein localization in the nuclei is not overlapped with centromere or histon H3K9me2/3 condense domain. Taken together, these results suggest that unlike HP1 of animals, plant TFL2 represses specific euchromatin genes but does not affect the gene expression or formation of heterochromatin.

Recently, genome-wide localization analyses of TFL2 and methylated histon markers were reported. It suggests that TFL2 co-localizes with H3K27me3 rather than H3K9me2/3, which is consistent with our previous results.