Retrotransposons: central players in the structure, evolution and function of plant genomes

One class of mobile DNA, the retrotransposons, constitutes a major portion of all eukaryotic nuclear genomes (often half of the total DNA), and has made a significant contribution towards the genome rearrangements that give rise to altered gene order and novel gene regulation. Research on plant retrotransposons is supported in only a handful of laboratories worldwide, but their progress continues to be striking, as shown at a recent workshop*.

Retrotransposons are Class I transposable elements, transposing via an RNA intermediate that is reverse transcribed into extrachromosomal DNA and inserted into the genome by the encoded reverse transcriptase, RNaseH and integrase enzymes. This replicative mode of transposition is similar to animal retroviruses. However, unlike the retroviruses, which are found only in animals, retrotransposons are found in all eukaryotes, where they are the most abundant class of mobile DNA. Many properties of transposable elements suggest that they are parasitic or selfish DNAs. However, like any other component of a heritable genome, retrotransposon DNAs can serve as the fodder for mutation and natural selection. Recent data have shown that they have played (and are playing) a central role in the evolution of gene function and genome structure. The talks presented at the workshop covered aspects of the regulation of transposition of both the long terminal repeats (LTR)-retrotransposons and non-LTR-retrotransposons in plant genomes, environmental correlations with the abundance of a barley LTR-retrotransposon and its contribution to genome size, bioinformatic analysis of plant transposable elements, the rates and specificities of retrotransposon amplification and mutation in maize, and retrotransposon-based marker technology for plant genome analysis. The following brief summary highlights some of the important points made in these presentations.

Regulation of transcription and transposition

Hirohiko Hirochika (National Institute of Agrobiological Resources, Tsukuba, Japan) presented evidence regarding the regulation of transcription and transposition in an LTR retrotransposon from tobacco, *Tto1*. The *Tto1* element belongs to the Ty1-*copia* group of LTR-retrotransposons and is present in a few hundred copies per tobacco genome. Transcription (and, hence, transposition) of the element is regulated by both abiotic and biotic stress conditions acting on *cis* elements within the 5′ LTR and adjacent untranslated regions. A detailed structural and functional analysis of the *Tto1* LTR promoter revealed a 13-bp repeated motif to be a positive *cis*-acting regulatory sequence that is involved in the expression of the element in protoplasts and in a callus as well as in wound- and methyl jasmonate-induced expression in leaves. Furthermore, a transcription factor, LBM1, identical to a previously reported MYB protein, was found to bind to the 13-bp motif. It appears that the activation of *cis*-elements by transcription factors involves a post-transcriptional event such as phosphorylation of transcriptional-acting factors. Hirochika also reported on the role of DNA methylation in the regulation of the *Tto1* element in transgenic plants[1]. Transgenic *Tto1* elements become silent after initial active retrotransposition in the *Arabidopsis* genome. When the silenced *Tto1* copies were introduced into the homozygous genetic background of a decreased DNA methylation mutant, *ddm1*, *Tto1* also became hypomethylated and its transcription and transposition were reactivated. These results provide clear evidence that the transposition of retrotransposons is controlled both by epigenetic mechanisms, such as those associated with viral defence, and by *cis*-regulatory motifs residing in the LTR promoters.

Jean-Marc Deragon (Université Blaise Pascal, Clermont-Ferrand, France) described the regulation of short interspersed nuclear elements (SINEs) in *Brassica* and *Arabidopsis*[2]. SINEs are ubiquitous components of complex animal and plant genomes, and are derived from RNA polymerase III (PolIII) genes. In spite of the presence of an active internal PolIII-promoter in most S1 copies, the transcription of S1 in *Brassica napus* is severely repressed. DNA methylation is associated with this repression. S1 generally inserts into hypomethylated DNA regions, but then becomes methylated. Hence, S1 elements do not simply adopt the methylation status of the surrounding regions but are directly targeted by methylases. Following integration, methylation can spread from the SINE element into flanking genomic sequences, creating distal epigenetic modifications. This spread of local methylation is preferentially directed either upstream or downstream of the S1 element, varying with the particular insertion site, suggesting a possible involvement of DNA replication. S1 transposition is also regulated at the post-transcriptional level. S1 RNA is cleaved to a smaller poly(A)-minus product by a process analogous to the maturation of mammalian SINEs. Because transposition appears to be limited to unprocessed transcripts, the formation of S1 poly(A)-minus RNA should lower the transpositional potential of S1 transcripts. Finally, S1 elements were shown to insert preferentially in matrix attachment regions (MARs). In an *in vitro* assay, S1 insertion often modified (usually decreased) the binding capacity of MARs. Although the biological implications of these events have not been assessed, the changes induced by SINE integration in MARs might allow for subtle modifications in the organization of chromatin domains.

Impact on the host genome

Alan Schulman (University of Helsinki, Finland) provided evidence regarding the dynamic nature of the barley genome with respect to retrotransposon activation by environmental stimuli. He reported that a family of LTR retrotransposon, *BARE-1*, which is an abundant (16 000 full-length copies but ~50 000 copies of the LTR) and active component of the barley genome, displays almost a threefold intraspecific copy number variation among the natural population of wild barley *Hordeum spontaneum*. This dramatic variation in the genome size within the barley population is the result of both active amplification and loses of the *BARE-1* family retrotransposons. More interestingly, a correlation was shown between the *BARE-1* copy number and genome size in wild barley and the sharply differing microclimates in Evolution Canyon near Mount Carmel, Israel. This is the first report in which a testable molecular mechanism linking habitat with retrotransposon activation in a natural population has been addressed.

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In a plenary presentation, Jeff Bennetzen (Purdue University, West Lafayette, IN, USA) described comparative genome organization studies in grasses with respect to the rates and specificities of retrotransposon amplification

*The workshop on 'Plant Retrotransposons' in the International Society of Plant Molecular Biology Congress, Quebec City, Canada, June 21, 2000.

and mutation. Although colinearity between genes was often seen in the sequence analysis of orthologous regions of the maize, sorghum, rice, barley and wheat genomes, the presence of transposable elements in the regions were highly variable. In maize, most LTR-retrotransposons appeared to be intermixed with genic regions, whereas in rice, sorghum and barley the genic regions were found to have a much higher gene density, and fewer retrotransposons. By studying the degree of LTR divergence within single elements (which usually have identical LTRs at the time of insertion), it was also shown that the LTR retrotransposons that make up >70% of the maize nuclear genome had arrived at these locations by insertion within the past 2 to 6 million years. In addition, the data indicated that highly repetitive LTR retrotransposons, unlike their many low-copy-number relatives, preferentially insert into each other rather than into genes, whereas the non-LTR retrotransposons in maize appeared to show a gene-specific insertion or retention specificity.

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Additionally, Thomas Bureau (University of McGill, Montreal, Canada) had been asked to discuss his research on Miniature Inverted Repeat Transposable Elements (MITEs), which belong to several families of Class II (DNA) transposable elements. However, in the spirit of the session, Bureau also described his discovery of two genes that had been acquired by an LTR retrotransposon of maize, *Bs1*. As with his MITE work, Bureau presented a powerful case for the value of data mining by a researcher who is an expert both in the biology of his system and in the informatics tools[3].

Retrotransposons as genetic tools

Amar Kumar (Scottish Crop Research Institute, Dundee, Scotland) highlighted newly developed retrotransposon-based marker technology in plants[4]. There are several advantages in using retrotransposons as molecular markers because they are ubiquitous in the plant kingdom and are normally present in high copy numbers as interspersed repetitive sequences in plant genomes. Another important property of retrotransposons is that they possess a replicative mode of transposition, therefore the insertions are mostly stable. This is essential for determining parental lineage data in any study of phylogenetic relationships. Molecular marker technology based on SNPs (single nucleotide polymorphisms) and SSRs (simple sequence repeats) are reversible, which limits their use for such studies. The best example of the use of retrotransposon-based markers in phylogenetic and biodiversity studies is from the genus *Pisum*[4].

In addition, retrotransposon-based markers are being used in genetic linkage mapping, high-density mapping in target regions, assessing genetic background in the backcross conversion programme, and for gene-specific markers. Finally, it is clear from the above and other published reports that retrotransposons are central players in the structure, evolution and function of plant genomes. Hence, retrotransposons might be selfish but they are certainly not junk. Indeed, retrotransposons cannot be entirely selfish because both host genome and scientists can use them for their own benefit.

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Scaling the soil–plant–atmosphere continuum: from physics to ecosystems

Plants lose water for carbon dioxide at an exchange rate as high as 400 molecules of water per molecule of carbon dioxide fixed. Consequently, plants must transport large quantities of water to grow, and water is a limiting resource in most natural and managed systems. The linked processes of water acquisition, transport and loss are thus crucial in both plant carbon gain and the global water cycle. The study of these processes is pan-disciplinary: spanning soil physics, fluid dynamics, plant physiology at cellular and organismic scales, and environmental biophysics. Perhaps because of this broad scope, several controversial issues have surfaced in recent years. These range from a questioning of the cohesion-tension mechanism to whether plant transport dynamics need to be incorporated into climate models. A recent symposium* highlighted advances across disciplines, and emphasized the synergy of work at different scales. Emerging from the symposium was an appropriately integrated view of the soil–plant–atmosphere continuum that promises to resolve controversy and identified essential areas for future research.

*Symposium: The Water Limitation: Issues in Plant, Community, and Ecosystem Water Use. Ecological Society of America Annual Meeting, Snowbird, UT, USA, 4–10 August 2000.

Xylem refilling, hydrogels and transport dynamics

Although the basic mechanism of water transport appears well in hand, the transport of water through dead xylem conduits is surprisingly dynamic. Two poorly understood aspects of this transport were the focus of an exciting presentation by Missy Holbrook (Harvard University, Cambridge, MA, USA) featuring novel techniques for studying xylem transport. Cavitated xylem conduits might be able to refill in spite of significant tensions in the surrounding transpiration stream. Magnetic resonance imaging (MRI) and single-vessel cannelation are being applied to answering if, and how, this refilling is occurring. A model