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Retrotransposon-Mediated Mutation of Rice Blast Resistance Gene *Pi-b*

Nam-Soo Jwa* and Yong-Hwan Lee[1]

Department of Molecular Biology, College of Life Science, Sejong University, Seoul 143-747, Korea
[1]School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea
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Plants may have evolved a system of disease resistance to variety of pathogens through a long period of coevolution with a wide array of microorganisms. The resistance is determined by the role of interaction between resistance (*R*) genes in the host and complementary avirulence (*AVR*) genes in the pathogen. These gene-for-gene interactions had been proposed by Flor (1956), and have been observed from the interactions between plants and a wide range of pathogens such as fungi, bacteria, viruses, nematodes and insects (Curte, 1986). First *AVR* gene had been identified by Staskawicz et al. (1984), which was isolated from *Psuedomonas syringae* pv. *glycinea*. The *AVR* gene successfully transformed susceptible soybean cultivars into their genome and they became avirulent. Importance of the genetic basis of plant *R* gene had been paid attention early by plant breeders who first recognized the entity of disease resistance genes, and they were often inherited by Mendelian way. Since the first *R* gene *PTO* had been cloned (Martin et al., 1993), the molecular basis of the resistance mechanism has been extensively studied for the development of a novel strategy for disease resistance.

Breeding for resistant varieties has been a major tool for controlling this disease (Bonman, 1992). However, frequent breakdown of resistance has been reported relatively short after the resistant cultivars released in the field (Kiyosawa, 1982). Much effort has been conducted to understand the mechanisms responsible for breakdown of resistance. The phenomenon of resistance-breakdown to date has been mostly explained by genetical changes in the fungal pathogens through heterokaryosis (Suzuki, 1965), parasexual recombination (Genovesi and Magill, 1976), and aneuploid (Kameswar Row et al., 1985). More recently, unstability of avirulence (*AVR*) genes of the fungal pathogen such as *AVR-Pita*, *AVR1-TSUY* and *PWL2* was sug-

*Corresponding author.
 Phone) +82-2-3408-3645, FAX) +82-2-3408-3661
 E-mail) nsjwa@sejong.ac.kr

gested as an important factor in virulence changes at the molecular level (Valent and Chumley, 1994). It was also reported that *M. grisea* race containing *AVR-Pita* gained virulence through mutations including deletions, point mutation and insertion of *Pot3* in the *AVR* gene (Valent and Chumley, 1994). However, the precise mechanism of resistance-breakdown in rice against fungal infections remains to be elucidated. Although several potential mechanisms for new race appearance have been proposed, little information is available on the mechanisms of resistance-breakdown in the plant.

Classification, and Dispersal of Transposon

Most eucaryotes have repetitive DNA sequences, the major portion of the genome, present either as tandem repeats or dispersed in the genome (Flavell, 1980). It is difficult to elucidate how they were introduced into the genome and spread into a large population. There is a hypothesis that certain DNAs spread successfully all over the chromosome, thus the host might evolve a mechanism to reduce or eliminate further spreading.

Some repetitive sequences are transposable elements (SanMiguel et al., 1996; Uozu et al., 1997), which are categorized into two major groups (Fig. 1): classes I and II (Finnegan, 1989; Kumar and Bennetzen, 1999). Class I includes retrotransposons and retroelements. Retrotransposons copy themselves through reverse transcription using an RNA intermediate. There are two different types of retrotransposons, one viral and the other non-viral retrotransposon families (Weiner et al., 1986). Non-viral retroransposons are grouped into non-LTR and LTR retrotransposons (Schmidt, 1999). Non-LTR retrotransposons are further distinguished into LINEs (long interspersed nuclear elements) and SINEs (short interspersed nuclear elements). LTR retrotransposons have flanking long terminal repeats and are divided into *Ty1-copia*-like and *Ty3-gypsy*-like retroele-
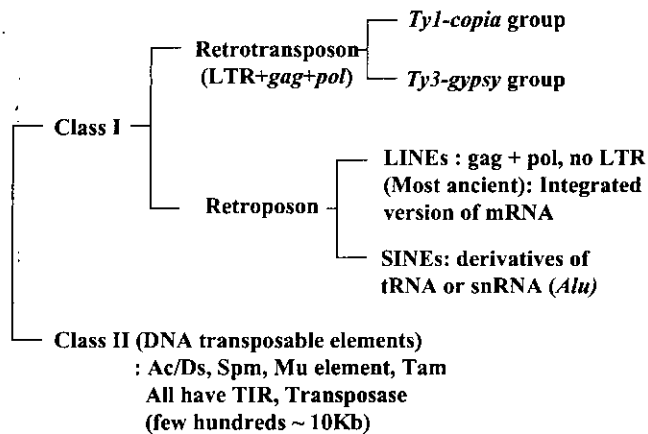
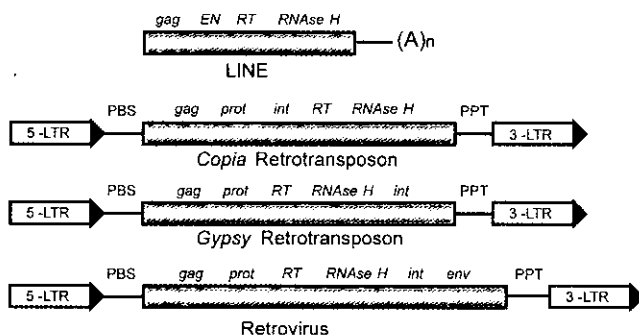Fig. 1. Classification of transposable elements.

Fig. 2. General structures of the LINE, *Ty1-copia*, *Ty3-gypsy*, and retrovirus as mobile DNA structures. The LTR retrotransposons have long terminal repeats in direct orientation at each end. The genes within the coding region encode *gag* protein (*gag*), protease (*prot*) reverse transcriptase (*RT*), RNase-H, and integrase (*int*). Other sequences featured are primer binding sites (PBS), polypurine tracts (PPT), 5'-UTR(5' untranstated region), and 3'-UTR (3' untranslated region). The envelope (*env*) gene-like sequence is present in plant retrovirus.

ments (Fig. 2). Class II elements move as DNA copies. This group includes *Ac/Ds*, *En/Spm*, and *Mu* transposons (Wessler, 1995).

There are many transposon families which have common sequences and are ubiquitous in plant genomes (Voytas et al., 1992). Computer-based search revealed previously described as well as newly discovered mobile element families have common sequences (Bureau et al., 1996). They appear to have been spread randomly all over the chromosomes without a hot spot through a specific insertion into the specific region. However, the function of the repetitive sequence remains unclear. Activation or movement of the transposon was investigated under various conditions. It has been reported that transposition and amplification of the retrotransposons could be induced through cell culture, wounding, infection by pathogen, and stress conditions (Grandbastien et al., 1989; Hirochika, 1993; Hirochika, et

al., 1996; Vernhettes, et al., 1997; Takeda et al., 1998). Microbial elicitors could activate Tn1 expression (Pouteau, 1994). Furthermore, the PCR-based survey showed evidences of the mobility of insertion elements in the wild-type rice. Transposition can be used for gene tagging and cloning of the mutated gene (Hirochika et al., 1996). Since there is a strong hypothesis that the 12 rice chromosomes may have originated from one or more of the ancient chromosomes (Wang et al., 1999b), retrotransposon-induced polymorphism during the evolution may be used as a marker for genotyping and linkage analysis (Grandbastien, 1989; Wang et al., 1997).

Transposons in the Rice Plant Genome

Since McClintock (1948) developed the concept of transposable elements as mobile genetic entities, numerous transposable elements from various plants were identified and characterized. Transposons alter their chromosomal locations and consequently often induce mutations by integrating into other genes and destroying their structural integrity (Gierl and Saedler, 1992; Hirochika, 1993; Hirochika et al., 1996). Several transposons such as a family of *Tos*, *copia*-like retrotransposon, p-SINE1, *RIRE1*, *RIRE2* have been identified in *Oryza sativa* and wild rice species (Hirochika et al., 1992; Wang et al., 1999a; Mochizuki et al., 1992; Noma et al., 1997; Ohtsubo et al., 1999). It has been suggested that some of the ancient transposable elements may have played an important role in the evolution of plant genomes by altering structures of coding regions, pattern of splicing, and regulation of gene expression (Finnegan, 1989; Weil et al., 1990; Wessler et al., 1995). The higher plant genome undergoes transposon-mediated structural changes during plant development as well as over evolutionary time period (Finnegan, 1989). Induction and insertion of retrotransposon *Tos17* were also activated in rice during tissue culture (Hirochika, 1997).

The genus *Oryza* has more than 20 species including 6 genomes, AA, BB, CC, BBCC, CCDD, EE, and FF with 2n = 24 or 48 (Vaughan, 1994). Various retrotransposons have been identified from *O. sativa* and wild rice species. *TrsB*, *TrsC*, and *RIRE1* have been previously identified in wild rice species using FF, CC, and EE genome, respectively (Nakajima et al., 1996). Retrotransposons family such as *Tos1*, *Tos2*, *Tos3*, *Tos4*, and *Tos5* were isolated from *O. sativa*. Wang et al. (1997) found *copia*-like retrotransposons using degenerated oligonucleotide primers. However, rice retrotransposon has not yet been well-characterized.

Contribution of Retrotransposons to Rice Blast Resistance Gene *Pi-b*

Rice blast, caused by *Magnaporthe grisea*, is one of the most devastating diseases throughout rice-growing regions in the world. Breeding for resistant varieties has been a major tool for controlling this disease (Bonman, 1992). However, frequent breakdown of resistance has been reported relatively short after the resistant cultivars released in the field (Kiyosawa, 1982). The precise mechanism of resistance-breakdown in rice against fungal infections remains to be elucidated. Although several potential mechanisms for new race appearance have been proposed, little information is available on the mechanisms of resistance-breakdown in the plant.

During the last decade, rapid technical advances in plant molecular genetics made it possible to study on the mechanism of resistance-breakdown in rice. Several resistance genes in rice were identified, mapped and characterized at the molecular level. Furthermore, accumulation of genome sequencing data helps to understand the structure and distribution of these genes in the rice genome. The rice blast resistance genes confer a high race-specific resistance to various races of blast fungi. The specific gene-for-gene interactions between resistance genes in rice and avirulence genes in the fungal pathogen can be explained by the gene-for-gene concept (Flor, 1956). The *Pi-b* is one of the resistance genes conferring resistance to rice blast fungus *M. grisea* race having an avirulence gene, *AVR-Pi-b*. This gene was originally found in *indica* cultivars and introgressed into *japonica* background on the telomere region of chromosome 2 (Yokoo et al., 1978). The *Pi-b* had been mapped with high resolution and cloned by positional cloning strategy (Miyamoto et al., 1996; Monna et al., 1997; Wang et al., 1999b).

To understand the molecular basis of the blast resistance provided by *Pi-b*, the *Pi-b* gene was cloned by map-based cloning strategy. During the *Pi-b* genomic sequencing, insertion sequences were identified along the coding region from susceptible cultivar, and that was believed to be the major cause of resistance-break down of the rice. The retrotransposon was cloned from the genomic DNA, characterized and designated as *Osr1*. One more copy of non-functional *Pi-b* was identified near the functional *Pi-b* gene from resistant cultivar, and there were two insertions along the coding and non-coding region of the non-functional. Among the insertion sequences, there was a 89 bases tandem repeat as well as retrotransposon which is not identified yet ·

Identification of a New Retrotransposon *Osr1* from *Pi-b* Gene

Insertion sequence was detected in the open reading frame of the *Pi-b* gene from the rice cv. Nipponbare, which is susceptible to rice blast fungi race *AVR-Pi-b*. The insertion sequence was identified as a solo long terminal repeat (LTR) of a new rice *Copia*-like retrotransposon and designated as *Osr1*. The *Osr1* showed a total of 6386 bp-nucleotide sequence including 965 bp LTRs on both ends with an 82% nucleotide sequence identity to the wheat *Tar1* retrotransposn on its reverse transcriptase. LTR was widely distributed in the rice genome. Various types of the restriction fragment length polymorphism (RFLP) of LTR were detected in *indica* cultivars, whereas only a few were detected in the *japonica* cultivars. The population of the *Osr1* is lower in the wild-type rice compared to that in the cultivated cultivars. Nucleotide divergence was noted among the individual LTRs as well as the coding region of *Osr1* through point mutations, small insertion or deletion. The insertion of LTR sequence in the *Pi-b* gene in the susceptible cultivar suggested that retrotransposon-mediated insertional mutation might play an important role in the resistance breakdown as well as the evolution of resistance genes in rice. This data also show that the *japonica* cultivars, which have been cultivated in the northeastern Asia including Korea, China, and Japan have high homogeneity in their genetic background. Northern blot data with LTR probes indicates that *Osr1* transposes through RNA intermediate to DNA and transcription was up-regulated through the inoculation of the rice blast fungus, *Magnaporthe grisea*.

Conclusions

New retrotransposon *Osr1* present in the rice genome was characterized through a direct comparison of the ORF of the *Pi-b* region from the susceptible and resistant rice cultivars against *M. grisea* infection. Only a solo LTR remained on *Pi-b* ORF, suggesting that the internal region of the retrotransposon was deleted with only a foot-print remaining. Complete unit of the retrotransposon was cloned by screening the rice genomic library with an LTR fragment as a probe. The size of the *Osr1* was shorter than RIRE1 which was the first identified and fully characterized retrotransposon from the wild rice *O. australiensis* (Noma et al., 1997). Nucleotide divergence was detected through a direct comparison with the LTRs of other clones selected from genomic library as well as the internal region obtained through a random amplification with PCR primers and subcloning. *Osr1* was the most similar to *Tar1* compared to the other homologous *Ty2-copia* group retrotransposons found so far from rice and other species based on the Genbank database (Fig. 3).

The distribution of *Osr1* was variable in the same genus *Oryza*. The population of the retrotransposon was significantly rare in the wild-type cultivars compared to that of the
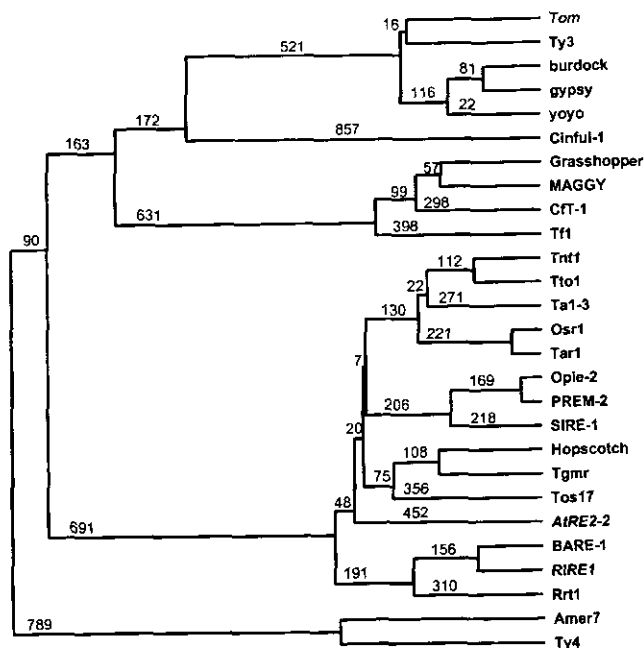
Fig. 3. Phylogenetic tree of revese transcriptase among *Copia*- and *Gypsy*-like retrotransposons. A tree was constructed by the UPGMA (unweighed pair group method,arithmetic means) using Genetyx program (Software Development, Tokyo, Japan). The number above horizontal line is the frequency with which a given branch appeared in 1000 bootstrap replications. Two groups belonging to *Copia*- and *Gypsy*-like retrotransposons were used. Tom (Z24451), Ty3 (M23367), burdock (U89994), gypsy (M12927), yoyo (U60529), Cinful-1 (AF049110), Grasshopper (M77661), MAGGY (L35053), CfT-1 (Z11866), Tf1 (M38526), Tnt1 (X13777), Tto1 (D83003), Ta1-3 (X13291), Osr1 (AB046118), Tar (AB008772), Opie-2 (U68408), PREM-2 (U41000), SIRE- (AF053008), Hopscotch (AW154801), Tgmr (U96748), Tos17 (D85876), AtRE2-2 (AB021267), BARE-1 (Z17327), RIRE1 (D85597), Rrt1 (Z75496), Amer7 (AJ006562), Ty4 (M94164).

Fig. 4. Evolution of rice blast resistance gene *Pi-b* into *indica* (resistant) and *japonica* (susceptible) cultivars. Two copies of *Pi-b* genes were identified in resistant cultivars (*Pi-b*, *Pi-b'*), and only one copy was detected in susceptible cultivars (*Pi-b"*).

cultivated rice cultivars including *indica* and *japonica* types. This result was similar to the cases of *RTL-1* and *RTL-2*, which were identified and characterized in *japonica* cultivar and detected only in AA genome wild-type rices (Wang et al., 1993).

Internal region of *Osr1* had a nucleotide heterogeneity (Fig. 2). It appeared that many LTR-retrotransposons in a plant were defective or existed as solo LTRs or with internal deletions, rearrangements, and replacements (Hu et al., 1995; Jin and Bennetzen 1994), which might have helped in escaping from being deleted out of the inserted site through the accumulation of random mutation. The high population of the repetitive sequences in rice suggests that these sequences have been active for a long period of time.

Transcriptional activation of the *Osr1* was detected after the infection with the blast fungus *M. grisea*, as reported in tobacco by Vernhettes et al. (1997). McClintock (1984) also suggested that stresses activate transposable elements and
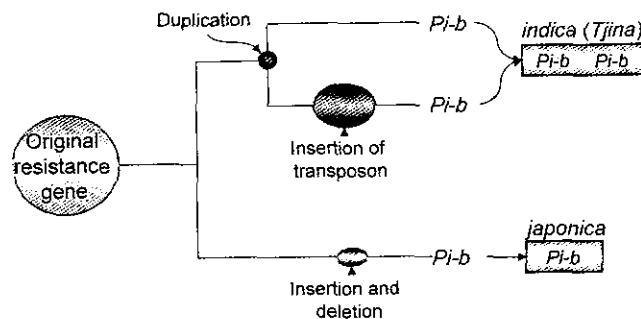
play an important role in generating new individual or species.

The insertional mutation was identified on the resistant and susceptible *Pi-b* genes (Jwa, 2000a). It is interesting to ponder whether the *Osr1*-mediated mutation occurred before or after the *japonica* cultivars were introduced into the northeastern Asia. There are two possibilities, the first being that the susceptible rice cultivar was introduced and adapted as the major cultivars and the second that the resistant rice was introduced into the non-stress environments where there were no *AVR-Pi-b* containing *M. grisea* races. Since mutant *Pi-b* rice could also have survived at that time due to the favorable microflora, susceptible rice cultivar could be the dominant variety in the northeastern Asia. Interestingly, the distribution of *Osr1* in the genome was different between susceptible *japonica* and resistant *indica* rice cultivars (Fig. 4). This signifies that the difference in the stresses such as races of the pathogens could change the type of mutation caused by retrotransposons. In conclusion, retrotransposon *Osr1* was identified as the major factor causing the insertional mutation on the rice blast resistant *Pi-b* gene, showing a RNA-to-DNA mediated transposition. Furthermore, mRNA transcripts were up-regulated through the infection with compatible and incompatible races of *M. grisea*. *Osr1* was randomly scattered in the rice genome, not located in a site-specific manner. Since RFLP pattern of *Osr1* was polymorphic between *japonica* and *indica* cultivars, the transposition of *Osr1* might play a role in the generation of diverse varieties of rice. Thus, understanding the role of transposition of retrotransposons in plant genome will help to elucidate the evolution and/or generation of various plant species.

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