Plant Signaling & Behavior 8:8, e25169; August 2013; © 2013 Landes Bioscience

The copy number of rice CACTA DNA transposons carrying *MIR820* does not correlate with *MIR820* expression

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Keywords: transposon, *Oryza sativa*, DNA methyltransferase, *miR820*, *OsDRM2*

miR820 is a small RNA species (22 and 24 nucleotides), produced from transcripts originated from a region inside CACTA DNA transposons in rice. Because *MIR820* is a transposon gene, its expression may depend on the transposon copy number. Here, we investigated the copy number of *MIR820* and its expression levels in various cultivars and wild species of rice. We found no correlation between copy number and expression level, suggesting that *MIR820* transcription is regulated not by the copy dosage but by the epigenetic state of each copy.

Transposable elements (TEs) and their remnants are the major components of eukaryotic genomes.[1] TEs have increased their copy number in the host genome because they replicate faster than the host genome. Thus, TEs are referred to as ultimate parasites that proliferate selfishly in the genome.[2,3] TE transposition induces insertion mutations and chromosome aberrations, causing instability of the host genome. Therefore, most TEs are kept silent by their host. Small RNA-mediated RNA silencing participates in suppression of TEs; this mechanism has a similar role in plants and animals.[4-6]

The prominent presence of TEs in the host genome suggests the existence of a running battle between the host defense machinery suppressing transposition of TEs and TEs' countermeasures against host-mediated silencing. However, little is known about the strategies that TEs have escaped the host silencing. We have previously shown that members of the *microRNA820* (*miR820*) family negatively regulate *OsDRM2* (a de novo DNA methyltransferase gene), which allows transposons to escape silencing by the host. We also found a dramatic proliferation of CACTA transposons carrying *MIR820* in some wild rice accessions, such as BB- and BBCC-genome species.[7] Therefore, we assumed that *MIR820* expression would be extremely high in these species.

To test this, we conducted northern blot analysis to detect *miR820* in three different cultivated rice accessions (*Oryza sativa*) and one wild rice species (*Oryza punctata*, accession W1514) (**Fig. 1A**). Surprisingly, no expression was detected in W1514 despite the presence of more than 18 copies of *MIR820*.[7] Accordingly, no cleavage of *OsDRM2* by *miR820* was detected in W1514 (**Fig. 1B**). Next, we determined the levels of *pre-miR820* by quantitative RT-PCR (qRT-PCR) in two *O. sativa* cultivars (AA) and four wild rice accessions belonging to two species containing the BB or BBCC genome, in which CACTA carrying *MIR820* is highly amplified (**Fig. 1C**).[7] The qRT-PCR revealed that the absence of *miR820* in W1514 is due to a reduced level of the *pre-miR820* transcript, rather than to its reduced processing. This implies that the high copy number of *MIR820* in wild rice accession with BB or BBCC genome may cause a stronger *MIR820* silencing in these species.

To explore whether the expression level of *MIR820* depends on its copy number, we also analyzed rice accessions with low or moderate copy numbers, as determined by Southern hybridization. We used 45 cultivars from the Japanese Rice Core Collection (JRC) and 56 cultivars from the World Rice Core Collection (WRC) (**Tables 1 and 2**).[8,9] Among the JRC cultivars, the average copy number of *MIR820* was 4.5 (minimum: 2; maximum: 6). Among the WRC cultivars, the average copy number was 6.3 (minimum: 3; maximum: 11) (**Fig. 2A**). Then, we determined the levels of *pre-miR820* by qRT-PCR in the same accessions and found no correlation between the *MIR820* copy number and *pre-miR820* levels (**Fig. 2B**).

Previously, we have shown that *miR820* downregulates the expression of de novo DNA methyltransferase, responsible for transposon inactivation.[7] Therefore, one would assume that once *miR820* effectively suppresses *OsDRM2*, silencing of transposons (including CACTA carrying *MIR820*) would be released, resulting in an increased transcription of *MIR820*. This would lead to a feed-forward loop, reinforcing the function of *miR820*. However, in this study, we found that the expression of *MIR820* is relatively constant in many cultivated rice cultivars, despite its copy number varying from 2 to 11. This suggests that *MIR820* transcription is regulated not by copy dosage, but rather by the epigenetic state of each locus. This mechanism may have evolved

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Submitted: 05/10/13; Revised: 05/23/13; Accepted: 05/24/13
Citation: Nosaka M, Ishiwata A, Shimizu-Sato, S, Ono A, Ishimoto K, Noda Y, Sato Y. The copy number of rice CACTA DNA transposons carrying ***MIR820*** does not correlate with ***MIR820*** expression. Plant Signal Behav 2013; 8: e25169; http://dx.doi.org/10.4161/psb.25169

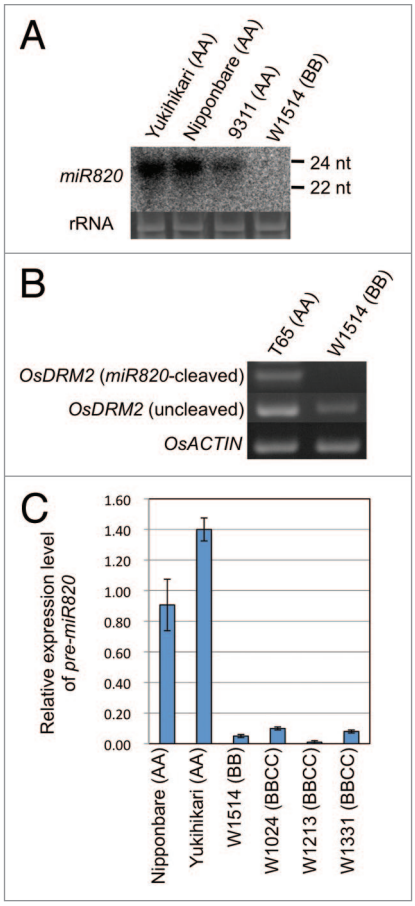

Figure 1. Expression and functional analysis of *miR820* in cultivated rice and wild rice species. (**A**) Northern blot analysis of *miR820* expression in AA and BB *Oryza* species. (**B**) Detection of *miR820*-cleaved *OsDRM2* mRNA by RNA ligation-mediated 5'-RACE (upper panel) in AA and BB species. The same cDNA templates were used for PCR to amplify uncleaved *OsDRM2* (middle panel) and *OsACTIN* (bottom panel) as controls. (**C**) The relative expression levels of pre-*MIR820* measured by qRT-PCR in AA, BB and BBCC *Oryza* species and calculated by subtracting the values with RT reaction by the values without RT reaction, then normalized by *OsACTIN*. Values are means of triplicate experiments, with bars showing standard errors. Probes and primers are described in our previous work.[7]

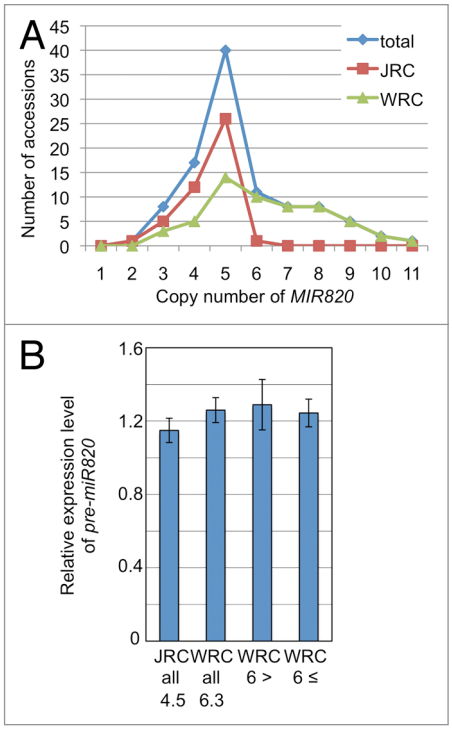

Figure 2. Copy numbers and expression levels of *MIR820* in the Japanese (JRC) and World (WRC) rice collections. (**A**) Distribution of the copy number of CACTA transposons carrying *MIR820* determined by Southern blot analysis. Genomic DNAs from the NIAS JRC and WRC rice core collections were digested with EcoRI and *Eco*RV and probed with the *pre-miR820* DNA fragment. "Total" is the combination of both collections. (**B**) The relative expression levels of *MIR820* measured by qRT-PCR as the same method mentioned before. The expression levels in all JRC and WRC accessions, in WRC accessions with less than six copies and those with six or more copies of CACTA are indicated. Numbers below JRC all or WRC all indicate the average copy numbers. Values are means of triplicate experiments, with bars showing standard errors. The numbers below JRC or WRC indicate the copy number of *MIR820*. Probes and primers are described in our previous work.[7]

during the "arms race" between the host and the parasite and may allow the host to inhibit the feed-forward loop triggered by *miR820* and thus to prevent the overwhelming victory of the parasites.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Ms. Tomoko Atsumi for technical assistance. The wild rice accessions and Core Collections of rice cultivars used in this study were obtained from the National Institute of Genetics, supported by the National Bioresource Project, MEXT and the Genebank at the National Institute of Agrobiological Sciences (NIAS), Japan. This work was supported by JSPS KAKENHI Grant 23658006 to Y.S.

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Table 1. Copy numbers of *MIR820* in the Japanese Rice Core Collection (JRC)

JRC No.	Name	Origin	Copy number
JRC01	Gaisen Mochi	Japan (unknown)	4
JRC03	Hinode	Kinki	5
JRC04	Senshou	Tokyo	4
JRC05	Yamada Bake	Kagoshima	4
JRC06	Kaneko B	Kantou Touzan	5
JRC07	Iruma Nishiki	Saitama	5
JRC08	Okka Modoshi	Japan (unknown)	4
JRC10	Hirayama	Tokyo	4
JRC11	Kahei	Kagoshima	4
JRC12	Oiran	Kumamoto	5
JRC13	Bouzu Mochi	Ooita	5
JRC17	Akage	Akita	4
JRC19	Wataribune	Shiga	3
JRC20	Hosogara	Aomori	4
JRC21	Akamai	Kouchi	5
JRC22	Mansaku	Nagano	5
JRC23	Ishijiro	Toyama	5
JRC24	Joushuu	Yamagata	5
JRC25	Dango	Japan (unknown)	5
JRC26	Aikoku	Fukui	5
JRC27	Ginbouzu	Ishikawa	5
JRC28	Shinriki Mochi	Kumamoto	5
JRC29	Shichimenchou Mochi	Japan (unknown)	5
JRC30	Morita Wase	Yamagata	5
JRC31	Kameji	Shimane	6
JRC32	Omachi	Okayama	5
JRC33	Shinriki	Hyougo	5
JRC34	Kyoutoasahi	Kyoto	5
JRC35	Kabashiko	Miyazaki	5
JRC37	Shinyamadaho 2	Hyougo	5
JRC38	Nagoya Shiro	Akita	2
JRC39	Shiroine	Tokushima	4
JRC40	Akamai	Nagasaki	5
JRC41	Akamai	Tokushima	3
JRC42	Touboshi	Kagoshima	5
JRC43	Akamai	Kantou Touzan	3
JRC44	Karahoushi	Kagoshima	3
JRC46	Fukoku	Hokkaido	5
JRC47	Okabo	Japan (unknown)	4
JRC48	Hakamuri	Kagoshima	4
JRC49	Rikutou Rikuu 2	Japan (unknown)	5
JRC51	Shinshuu	Nagano	5
JRC52	Aichiasahi	Aichi	3
JRC53	Raiden	Kantou Touzan	5
JRC54	Houmanshinden Ine	Kagoshima	4

Table 2. Copy numbers of *MIR820* in the World Rice Core Collection (WRC)

WRC No.	Name	Origin	Copy number
WRC01	Nipponbare	Japan	5
WRC02	Kasalath	India	6
WRC03	Bei Khe	Cambodia	6
WRC04	Jena 035	Nepal	5
WRC05	Naba	India	8
WRC06	Puluik Arang	Indonesia	3
WRC07	Davao 1	Philippines	3
WRC09	Ryou Suisan Koumai	China	5
WRC10	Shuusoushu	China	9
WRC11	Jinguoyin	China	5
WRC12	Dahonggu	China	7
WRC13	Asu	Bhutan	6
WRC14	IR 58	Philippines	4
WRC15	Co 13	India	7
WRC16	Vary Futsi	Madagascar	7
WRC17	Keiboba	China	11
WRC18	Qingyu (Seiyu)	China	8
WRC19	Deng Pao Zhai	China	4
WRC20	Tadukan	Philippines	6
WRC21	Shwe Nang Gyi	Myanmar	6
WRC22	Calotoc	Philippines	8
WRC23	Lebed	Philippines	5
WRC24	Pinulupot 1	Philippines	5
WRC25	Muha	Indonesia	10
WRC26	Jhona 2	India	10
WRC27	Nepal 8	Nepal	9
WRC28	Jarjan	Bhutan	7
WRC29	Kalo Dhan	Nepal	7
WRC30	Anjana Dhan	Nepal	5
WRC31	Shoni	Bangladesh	6
WRC32	Tupa 121–3	Bangladesh	6
WRC33	Surjamukhi	India	9
WRC34	ARC 7291	India	9
WRC35	ARC 5955	India	9
WRC36	Ratul	India	7
WRC37	ARC 7047	India	8
WRC39	Badari Dhan	Nepal	8
WRC40	Nepal 555	India	6
WRC41	Kaluheenati	Sri Lanka	5
WRC42	Local Basmati	India	5
WRC43	Dianyu 1	China	4
WRC44	Basilanon	Philippines	6
WRC45	Ma sho	Myanmar	6
WRC46	Khao Nok	Laos	5
WRC47	Jaguary	Brazil	8
WRC48	Khau Mac Kho	Vietnam	8
WRC49	Padi Perak	Indonesia	8
WRC50	Rexmont	USA	5
WRC51	Urasan 1	Japan	7
WRC52	Khau Tan Chiem	Vietnam	3
WRC53	Tima	Bhutan	5
WRC55	Tupa729	Bangladesh	7
WRC57	Milyang 23	Korea	4
WRC98	Deejiaohualuo	China	4
WRC99	Hong Cheuh Zai	China	5
WRC100	Vandaran	Sri Lanka	5

Table 2. Copy numbers of *MIR820* in the World Rice Core Collection (WRC)