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**Final Technical Report: Grant # DE-FG02-94ER20135
Structure, Regulation and Evolution of the R Transcriptional Activators from
Maize and Rice
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This grant provided the support for the publications that are listed (and summarized) below.

- 1. Wang, L. and Wessler, S.R. (1998) Inefficient reinitiation is responsible for upstream open reading frame-mediated translational repression of the maize *R* gene. *The Plant Cell* 10: 1733-1745.**

Maize *R* genes encode a small family of transcriptional activators of several structural genes in the anthocyanin biosynthetic pathway. The 5' leader region of most *R* genes contains a 38-codon upstream open reading frame (uORF) that previously was shown to be responsible for the repression of downstream gene expression in a transient transformation assay. In this study, we report that the 5' leader also can repress translation of the downstream luciferase gene both in the rabbit reticulocyte translation system and in transgenic rice plants. The ability to visualize the uORF peptide after in vitro translation permits quantification of both products of dicistronic mRNAs. Similarly, the construction of transgenic rice plants expressing wild-type and mutant constructs permits the quantification and correlation of steady state mRNA levels and reporter gene activities. Using these assays, we demonstrate directly that translation of the uORF is required for repression, that increasing translation of the uORF peptide decreases downstream gene expression, and that repression is unaffected by either subtle or gross changes in the uORF peptide. Rather, we find that ribosomes that translate the uORF reinitiate inefficiently and that the intercistronic sequence downstream of the uORF mediates this effect.

- 2. Wang, L. and Wessler, S.R. (2001) Role of mRNA secondary structure in translational repression of the maize transcriptional activator *Lc*. *Plant Physiol.* 125: 1380-1387.**

In this study, we report that a potential hairpin structure near the 5' end of the *Lc* mRNA also represses downstream translation in the rabbit reticulocyte in vitro translation system and in transient transformation assays. Base pairing of the hairpin is important for repression because its destabilization increases translation of the uORF and the downstream ORF. However, translation of the uORF is not required for the hairpin-mediated repression. Instead, the uORF and the 5'-proximal hairpin mediate two independent levels of repression. Although the uORF represses downstream translation due to inefficient reinitiation of ribosomes that translate uORF, the hairpin inhibits ribosome loading at the 5' end of the mRNA.

- 3. Hu, J., Reddy, V. and Wessler, S.R. (2000) The rice *R* gene family: two distinct subfamilies containing several miniature inverted-repeat transposable elements. *Plant Mol. Biol.* 42: 667-678.**

The *R* and *B* genes of maize regulate the anthocyanin biosynthetic pathway and

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constitute a small gene family whose evolution has been shaped by polyploidization and transposable element activity. To compare the evolution of regulatory genes in the distinct but related genomes of rice and maize, we previously isolated two *R* homologues from rice (*Oryza sativa*). The *Ra1* gene on chromosome 4 can activate the anthocyanin pathway, whereas the *Rb* gene, of undetermined function, maps to chromosome 1. In this study, rice *R* genes have been further characterized. First, we found that an *Rb* cDNA can induce pigmentation in maize suspension cells. Second, another rice *R* homologue (*Ra2*) was identified that is more closely related to *Ra1* than to *Rb*. Domesticated rice and its wild relatives harbor multiple *Ra*-like and *Rb*-like genes despite the fact that rice is a true diploid with the smallest genome of all the grass species analyzed to date. Finally, several miniature inverted-repeat transposable elements (MITEs) were found in *R* family members. Their possible role in hastening the divergence of *R* genes is discussed.

4. Jiang, N. and Wessler, S.R. (2001) Insertion preference of maize and rice MITEs as revealed by the analysis of nested elements. *The Plant Cell* 13: 2553-2563.

A 128-bp insertion into the maize *waxy-B2* allele led to the discovery of *Tourist*, a family of miniature inverted repeat transposable elements (MITEs). As a special category of nonautonomous elements, MITEs are distinguished by their high copy number, small size, and close association with plant genes. In maize, some *Tourist* elements (named *Tourist-Zm*) are present as adjacent or nested insertions. To determine whether the formation of multimers is a common feature of MITEs, we performed a more thorough survey, including an estimation of the proportion of multimers, with 30.2Mb of publicly available rice genome sequence. Among the 6600 MITEs identified, 10% were present as multimers. The proportion of multimers differs for different MITE families. For some MITE families, a high frequency of self-insertions was found. The fact that all 340 multimers are unique indicates that the multimers are not capable of further amplification.

5. Jiang, N., Bao, Z., Temnykh, S., Cheng, Z., Jiang, J., Wing, R.A., McCouch, S.M., Wessler, S.R. (2002) *Dasheng*: a recently amplified non-autonomous LTR element that is a major component of pericentromeric regions in rice. *Genetics* 161: 1293-1305.

A new and unusual family of LTR elements, *Dasheng*, has been discovered in the genome of *Oryza sativa* following database searches of 100 Mb of rice genomic sequence and 78 Mb of BAC-end sequence information. With all of the *cis*-elements but none of the coding domains normally associated with retrotransposons (e.g., *gag*, *pol*), *Dasheng* is a novel nonautonomous LTR element with high copy number. Over half of the 1000 *Dasheng* elements in the rice genome are full length (5.6–8.6 kb), and 60% are estimated to have amplified in the past 500,000 years. Using a modified AFLP technique called transposon display, 215 elements were mapped to all 12 rice chromosomes. Interestingly, more than half of the mapped elements are clustered in the heterochromatic regions around centromeres. The distribution pattern was further

confirmed by FISH analysis. Despite clustering in heterochromatin, *Dasheng* elements are not nested, suggesting their potential value as molecular markers for these marker-poor regions. Taken together, *Dasheng* is one of the highest-copy-number LTR elements and one of the most recent elements to amplify in the rice genome.

6. Jiang, N., Jordan, I.K. and Wessler, S.R. (2002) *Dasheng* and *RIRE2*: a non-autonomous LTR element and its putative autonomous partner in the rice genome *Plant Physiology* 130: 1697-1705.

Dasheng is one of the highest copy number long terminal repeat elements and one of the most recent elements to amplify in the rice (*Oryza sativa*) genome. However, the absence of any significant coding capacity for retroviral proteins, including *gag* and *pol*, suggests that *Dasheng* is a nonautonomous element. Here, we have exploited the availability of 360 Mb of rice genomic sequence to identify a candidate autonomous element. *RIRE2* is a previously described *gypsy*-like long terminal repeat retrotransposon with significant sequence similarity to *Dasheng* in the regions where putative cis factors for retrotransposition are thought to be located. *Dasheng* and *RIRE2* elements have similar chromosomal distribution patterns and similar target site sequences, suggesting that they use the same transposition machinery. In addition, the presence of several *RIRE2-Dasheng* element chimeras in the genome is consistent with the copackaging of element mRNAs in the same virus-like particle. Finally, both families have recently amplified members, suggesting that they could have been coexpressed, a necessary prerequisite for *RIRE2* to serve as the source of transposition machinery for *Dasheng*. Consistent with this hypothesis, transcripts from both elements were found in the same expressed sequence tag library.

7. Wessler, S.R., Nagel, A. & Casa, A. (2002) Miniature inverted repeat transposable elements help create genomic diversity in maize and rice. *Proceedings of the Fourth International Rice Genetics Symposium*. Eds. G.Khush, D. Brar, R. Hardy. 107-116.

This is a review of previously published data. .