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Roles of MAR and Plastid-Targeting Signal in Transgene Expression of Rice

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Improvements in transformation technology has allowed novel approaches for studying gene expression and opened new avenues for genetic modification of crop plants. To develop systems for secure and stable transgene expression, we investigated roles of the *rbcS* chloroplast targeting signal and the chicken lysozyme matrix attachment region (MAR) sequence in transgenic rice plants. Several fertile transgenic plants, that contain the *Act1-sgfp* for an untargeted and the *rbcS-Tp-sgfp* for a chloroplast targeted expression, were produced by the *Agrobacterium*-mediated method. Confocal microscopy, Nouthern blot and Western blot analyses demonstrated that the *rbcS* targeting signal not only localized GFP spcifically to chloroplasts and proplastids, but also increased levels of transgene products significantly yielding about 10% of the total soluble protein. Mutation in the targeting signal abolished the localization of transgene product and reduced the expression level, indicating correlation between targeting and high-levels of expression. MARs may function as domain boundaries and partition chromosomes into independently regulated units. The MAR sequence from the chicken lysozyme locus was tested for its effects on expression of a linked reporter gene sgfp in transgenic rice. Several fertile transgenic rice plants were produced by the Agrobacterium-mediated method. Southern, Nouthern and immunoblot analysis of 60 independent transgenic lines demonstrated that, in the absence of MAR, transgene expression levels markedly varied with the integration sites on chromosomes, whereas, in the presence of MAR, the levels were similar among different lines, suggesting position-independent expression. Moreover, the MAR sequence appears to confer copy number-dependence in gene expression. Potential use of our systems will be discussed