Corn Storage Protein - A Molecular Genetic Model

Final Report of DE-FG05-85ER13367

from 8/1/85 - 7/31/96

Joachim Messing

1. Summary

We have focused on the regulation of zein gene expression in the endosperm of different inbred lines (see storage proteins under publications) and to a smaller degree on technology development (see methods, vectors, and viruses under Publications). Our results can be divided into four areas: a) We have been the first group to clone and characterize the so called δ -zein genes. b) We have developed a tissue-specific transient expression system to study zein promoters. c) We have studied post-transcriptional regulation of gene expression and proposed a new model of 3' end processing in plants. d) We have been the first group to discover genomic printing of zein genes. Technology development included a) the improvement of the M13mp and pUC vectors system for DNA sequencing and mutagenesis, b) the improvement of cDNA cloning techniques, c) the first plant cDNA cloning by complementation, and d) the development of plant viral vectors.

2. The delta zeins

The delta zeins have been the last class of zein genes that have been cloned (Kirihara et al., 1988; Anderson Kirihara et al., 1988; Swarup et al., 1995). We have focused on those because the inbred line BSSS53 has a high concentration of the 10-kDa δ zein and gives rise to lysine/threonine resistance during germination, indicating a high level of protein-bound methionine. Variability in seed-methionine levels, however, not only provide an opportunity to study tissue-specific regulation of gene expression, but also to correct a nutritional deficiency in animal feed, where methionine is the only essential amino acid that is supplemented (Messing and Fisher, 1993). We were able to show that variability of seed methionine was due to the post-transcriptional regulation of the 10-kDa zein gene (Cruz-Alvarez et al., 1991; Schickler et al., 1993) by alleles of a trans-acting locus *dzr1* (Benner et al., 1989; Chaudhuri and Messing, 1995).

3. Zein promoters

The α zein genes are multigenic and even clustered in the same chromosomal location (Heidecker et al., 1991; Chaudhuri and Messing, 1995). However, many of them are not active due to in-frame stop codons. Others like the 27-kDa zein gene, a γ zein, are not multigenic and provide high level of expression. To study their promoter activity in a transient expression system requires formation of protoplasts from maize endosperm, where gene constructs can be introduced by electroporation (Ueda and Messing, 1991). As predicted from gene dosage *in vivo*, the 22-kDa promoters, an α zein, are weaker in the transient expression system than the 27-kDa zein promoter. Using promoter deletion experiments, a -300 element of the 27-kDa zein promoter has been characterized to be a critical enhancer region for zein gene expression (Ueda et al., 1994). Another element in the promoter region involves the ACGT core motif that in many promoters is recognized by b-zip transcription factors. We have used the transient expression system of maize endosperm protoplasts to study the interaction of the O2 protein, which is a b-zip transcription factor, with its target genes (Ueda et al., 1992).

4. Post-transcriptional regulation

Allelic variants of the 27-kDa zein gene exhibit different expression levels due to post-transcriptional regulation (Das and Messing, 1987). Since it has been known for many years that plant mRNAs deviate from animal mRNAs in their poly-A sites, any future

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

studies on mRNA accumulation will need a better understanding of the differences between plant and animal 3' end mRNA processing. Using the same transient expression system as well as a transient expression system of nondifferentiated maize and tobacco cells, we have shown that the preferential poly-A site in the 27-kDa zein gene is AATGAA rather than AATAAA. The 3' proximal site is used, but can be substituted by a second upstream AATGAA. The GT element, found downstream of the processing site, is not required, in contrast to animal genes. On the other hand, upstream sequences and their spatial arrangement are critical for the selection of the poly-A site and efficient 3' end processing. Furthermore, these signals are not tissue-specific and are conserved between tobacco and maize cells (Wu et al., 1993; 1994).

5. Allele-specific genomic imprinting of the 10-kDa δ zein

Whereas our studies of the regulation of the 27-kDa zein gene have focused mostly on *cis*-acting regulatory elements, the studies of the regulation of the 10-kDa zein gene have focused on the trans-acting factor that gives rise to the high-methionine, HM, phenotype. Most variants, defective in regulating storage protein synthesis like *opaque-2* and *floury-2*, have a broad effect on many genes. However, *dzrl*, on the short arm of chromosome 4 seems to affect only the relative expression of the 10-kDa δ zein gene (Benner et al., 1989; Chaudhuri and Messing, 1995). Moreover, we have identified different alleles of this regulator. One of them, *dzrl+Mo17*, shows two interesting differences compared to the *dzrl+BSSS53* allele that produces the highest level of 10-kDa protein (HM phenotype), perhaps by protecting the mRNA from turnover (Cruz-Alvarez et al., 1991). The Mo17 allele acts like a dominant negative mutant by repressing the overexpression of the 10 kDa zein gene (Schickler et al., 1993). The second difference is even more striking. Expression of this regulatory gene is silenced when it is transmitted through the male, but activated by passage through the female parent. If the mode of transmission is altered in the next generation, a switch occurs, indicating that the expression potential depends only on the immediate parentage. This is in contrast to *dzrl+W64A*, which exhibits a dosage effect in crosses with *dzrl+BSSS53*. Besides the *R* locus in maize, *dzrl* is the second example of allele-specific genomic imprinting in plants (Chaudhuri and Messing, 1994).

6. Publications

a. Methods, vectors, and viruses:

- 1) Vieira, J. and Messing, J. (1987). Production of single-stranded plasmid DNA. *Methods Enzymol.* 153, 3-11.
- 2) Snustad, D.P., Hunsperger, J.P., Chereskin, B.M. and Messing, J. (1988). Maize glutamine synthetase cDNAs: isolation by direct genetic selection. *Genetics* 120, 1111-1124.
- 3) Messing, J. and Bankier, A.T. (1989). The use of single-stranded DNA phage in DNA sequencing. In *Nucleic Acids Sequencing: A Practical Approach*. E.S. Ward and C.J. Howe, eds. IRL Press Ltd., Oxford, England, 1-36.
- 4) Das, O.P., Cruz-Alvarez, M., Chaudhuri, S. and Messing, J. (1990). Molecular methods for genetic analysis of maize. *Methods Mol. Cell. Biol.* 1, 213-222.
- 5) Ugaki, M., Ueda, T., Timmermans, M., Vieira, J., Elliston, K., and Messing, J. (1991). Replication of a geminivirus derived shuttle vector in maize endosperm cells. *Nucl. Acids Res.* 19, 371-377.
- 6) Vieira, J. and Messing, J. (1991) New pUC derived cloning vectors with different selectable markers and replication origins. *Gene* 100 189-194.
- 7) Messing, J. (1996). Cloning Single-Stranded DNA. *Molecular Biotechnology* 5, 39-47.

b. Reviews of plant gene structure:

- 1) Elliston, K. and Messing, J. (1988). The molecular architecture of plant genes: a phylogenetic perspective. In *Architecture of Eukaryotic Genes*. G. Kahl, ed. VCH Verlagsgesellschaft, Frankfurt, West Germany, 21-56.
- 2) Elliston, K. and Messing, J. (1988). The molecular architecture of plant genes: a look at the cellular level. In *Plant Biotechnology*. S.D. Kung and C.J. Arntzen, eds. Butterworths, Stoneham, MA, 115-139.
- 3) Elliston, K.O., Imran, S. and Messing, J. (1988). Cloned Genes of Storage Proteins. *Plant Mol. Biol. Rep.* 6, 22-26.
- 4) Messing, J. (1989). Broadening our understanding of genetics information: beyond base pairing. *ASM News* 55, 255-258.
- 5) Ueda, T. and Messing, J. (1993). Manipulation of amino acid balance in maize seeds. *Genetic Engineering* 2 15, 109-130.

c. Storage proteins:

- 1) Das, P. and Messing, J. (1987). Allelic variation and differential expression at the 27kD zein locus in maize. *Mol. Cell. Biol.* 7, 4490-4497.
- 2) Kirihaara, J.A., Hunsperger, J.P., Mahoney, W.C. and Messing, J. W. (1988). Differential expression of a methionine-rich storage protein gene in maize. *Mol. Gen. Genet.* 211, 477-484.
- 3) Anderson Kirihaara, J., Petri, J.B. and Messing J. (1988). Isolation and sequence of a gene encoding a methionine-rich 10-kDa protein from maize. *Gene* 71, 359-370.
- 4) Benner, M.S., Phillips, R.L., Kirihaara, J.A. and Messing, J.W. (1989). Genetic analysis of methionine-rich storage protein accumulation in maize. *Theor. Appl. Genet.* 78, 761-767.
- 5) Cruz-Alvarez, M., Kirihaara, J.A. and Messing, J. (1991). Post-transcriptional regulation of methionine content in maize kernels. *Mol. Gen. Genet.* 225, 331-339.
- 6) Ueda, T. and Messing, J. (1991). A homologous expression system for cloned zein genes. *Theor. Appl. Genet.* 82, 93-100.
- 7) Heidecker, G., Chaudhuri, S., and Messing, J. (1991). Highly clustered zein gene sequences reveal evolutionary history of the multigene family. *Genomics* 10, 719-732.
- 8) Messing, J. and Fisher, H. (1991). Maternal effect on high methionine levels in hybrid corn. *J. Biotechnol.* 21, 229-238.
- 9) Ueda, T., Wawerczak, W., Ward, K., Sher, N., Ketudat, M., Schmidt, R., and Messing, J. (1992). Mutations of the 22- and 27-kD zein promoters affect transactivation by the opaque-2 protein. *The Plant Cell* 4, 701-709.
- 10) Schickler, H., Benner, M., and Messing, J. (1993). Repression of the high-methionine zein gene in the maize inbred line Mo17. *The Plant Journal* 3, 221-229.
- 11) Wu, L., Ueda, T., and Messing, J. (1993). 3'-end processing of the maize 27 kDa zein mRNA. *The Plant Journal* 4, 535-544.
- 12) Chaudhuri, S. and Messing, J. (1994). Allele-specific parental imprinting of *dzr1*, a post-transcriptional regulator of zein accumulation. *Proc. Natl. Acad. Sci.* 91, 4867-4871.
- 13) Ueda, T., Wang, Z., Pham, N., and Messing, J. (1994). Identification of a transcriptional activator-binding element in the 27-kilodalton zein promoter, the -300 element. *Mol. Cell. Biol.* 14, 4350-4359.
- 14) Wu, L., Ueda, T. and Messing, J. (1994). Sequence and spatial requirements for the tissue- and species-independent mRNA 3'-end processing mechanism of plant mRNA. *Mol. Cell. Biol.* 14, 6829-6838.
- 15) Chaudhuri, S. and Messing, J. (1995). RFLP mapping of the maize *dzr1* locus that regulates methionine-rich 10-kDa zein regulation. *Mol. Gen. Genet.* 246, 707-715.
- 16) Wu, L., Ueda, T., and Messing, J. (1995). The formation of mRNA 3' ends in plants. *The Plant Journal* 8, 323-329.

- 17) Swarup, S., Timmermans, M.C.P., Chaudhuri, S., and Messing, J. (1995). Determinants of the high-methionine trait in wild and exotic germplasm may have escaped selection during early cultivation of maize. *The Plant Journal* 8, 359-368.