

MOLECULAR ANALYSIS OF A SUNFLOWER GENE ENCODING A HOMOLOGUE OF THE B SUBUNIT OF A CCAAT-BINDING FACTOR

SANI E.***, PISTELLI L.***, MICHELOTTI V.*, FAMBRINI M.*, PUGLIESI C.*,
SALVINI M.***

*) Dipartimento di Biologia delle Piante Agrarie, Sezione di Genetica, Università di Pisa, Pisa

***) Scuola Normale Superiore, Pisa

***) Dipartimento di Biologia delle Piante Agrarie, Sezione di Fisiologia Vegetale, Università di Pisa, Pisa

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The *Helianthus annuus* *LEAFY COTYLEDON1-LIKE* (*HaL1L*) gene encodes an NF-YB (or HAP3) of a CCAAT box-binding factor (NF-Y). The peptide HaL1L results homologous of the LEC1-LIKE of *A. thaliana*, sharing a high amino acid sequence identity (56%). *HaL1L* transcripts are accumulated primarily at an early stage of sunflower embryogenesis. High levels of *HaL1L* mRNA have been detected in the developing embryo proper, suspensor, endosperm, integument, and integumentary tapetum cells, while no or low transcript levels were detectable in organs such as the cotyledons, leaves, stem internodes, roots, and unfertilized ovules (Fambrini et al., 2006 Dev Genes Evol 216: 253-264). A large insert genomic library from *H. annuus* was successfully screened to isolate the entire *HaL1L* gene. From GenBank databases analyses it has been suggested that the identified genomic DNA fragment is homologous to the *A. thaliana* chromosome V region carrying *AtL1L* and the immediately adjacent genes at the 5' and 3' sides respectively. In the *HaL1L* 5' flanking region, elements peculiar to a putative TATA-box promoter and two "CG isles" were identified. An investigation on the methylation status of the CG rich DNA regions shows that differentially methylated cytosines are recognizable in DNA of embryos at the fifth day from pollination (DAP) in comparison to the leaf DNA. These data suggest an epigenetic regulation of *HaL1L* transcription carried out by methylation of cytosine residues during plant development. The observation that *HaL1L* mRNA is downregulated in leaf tissues and reach the higher steady state level in 5-DAP embryos support the results of methylation analyses (Fambrini et al., 2006). The nucleotide sequences were also analyzed to individuate *cis*-regulatory sequences involved in the *HaL1L* transcription regulation by other transcription factors (Yamamoto et al., 2007 BMC Genomics, 8: 67-90). One of the most intriguing motifs, present in the 5' flanking region as well in the *HaL1L* intron, is WUSATAg. It represent the target sequence for the transcription factor WUSCHEL (WUS) (Mayer et al., 1998 Cell, 95: 805-815), which could be involved in the complex regulation system controlling the zygotic embryo development. As regard to the 3' region, in addition to the nuclear polyadenylation signal, a cytoplasmic polyadenylation signal which suggest a negative post-transcriptional regulation was also identified. Poly(A) tails, lengthened by cytoplasmic poly(A) polymerases (PAPs), form complexes with regulative proteins which inactivate mRNAs. During embryo development PAPs act under hormonal control (Rothnie, 1996 Plant Mol Biol, 32: 43-61). Noteworthy, the presence of *ARF* and *ABRE* motifs in the *HaL1L* promoter region suggests auxin and abscisic acid involvement in the expression control of this gene. The hypothesis of a translational control for *HaL1L* is also supported by the *in situ*

hybridization analysis (Fambrini et al., 2006), that demonstrate an accumulation of *HaLIL* transcripts in maternal tissues of developing embryos such as integument and integumentary tapetum cells. On the basis of our study a control of *HaLIL* expression mediated at transcriptional level by both methylation of cytosine residues and interaction with other transcription factors is suggested. In addition, a control at translational level by a temporary unavailability of pre-synthesized *HaLIL* mRNA could be also supposed.