77

BARD

FINAL REPORT

PROJECT NO. IS-2120-92

**Regulation of Transposable Element Activities during
Plant Development**

A.A. Levy, V. Walbot

1996

Date: 9.12.96

BARD Project Number: IS 2120-92

Project Title:

Regulation of transposable element activities during plant development

Investigators:
(Principal first)

AVRAHAM LEVY

VIRGINIA WALBOT

Affiliated Institution:

Weizmann Institute

Stanford University

Project Start Date: Aug. 1992

Type of Report:

1st Annual

2nd Annual

Final X

Signature:
Principal Investigator

A. Levy

Signature:
Authorizing Official, Affiliated Institution

THE WEIZMANN INSTITUTE OF SCIENCE

T. Geles

Abstract

Regulation of transposable element activities during plant development

We have studied the regulation of the maize *Ac* and *MuDR* transposable elements activities during plant development. *Ac* was studied in an heterologous system (transgenic tobacco plants and cell suspensions) while *MuDR* was studied in the native maize background. The focus of this study was on the transcriptional regulation of *Ac* and *MuDR*. For *Ac*, the major achievements were to show that 1- It is autoregulated in a way that the *Ac*-encoded transposase can repress the activity of its own promoter; 2- It is expressed at low basal level in all the plant organs that were studied, and its activity is stronger in dividing tissues -- a behaviour reminiscent of housekeeping genes; 3- the activity of *Ac* promoter is cell cycle regulated -- induced at early S-phase and increasing until mitosis; 4- host factor binding sites were identified at both extremities of *Ac* and may be important for transposition. For *MuDR*, It was shown that it encodes two genes, *mudrA* and *mudrB*, convergently transcribed from near-identical promoters in the terminal inverted repeats. Distinct 5' start sites, alternative splicing, production of antisense RNA and tissue specificity were all shown to be involved in the regulation of *MuDR*.

Original Research Objectives

Our original objectives were to study the regulation of *Ac* and *Mu9* transposition throughout development. This included studies on the transcriptional regulation of transposase production and on the regulation of excision throughout development. We have focused on the first regulatory aspect, i.e. the transcriptional regulation as it proved to be complex in both *Ac* and *Mu9*.

Relevant data, methodology, results and discussion

1. Regulation of *Ac* promoter

Regulation of transposable elements (TEs) activity is a complex process which is affected by the element itself and by the host. The transcriptional regulation of *Ac* and *Mu9* was studied in transient assays and in transgenic plants.

1.1 Autoregulation of *Ac* promoter by its own encoded TPASE - Does the TPASE binding to the *Ac* 5' region contributes to transcriptional regulation of the element?

Our results on this topic have been published in the enclosed article: Fridlender M., Harrison K., Jones J.D.G. and Levy A.A. (1996). *The Plant Journal* 9: 911-917.

The main results reported in this manuscript are as follows: *Ac* encodes for one protein, the transposase (TPASE), which is necessary for transposition. Binding of TPASE to *Ac* termini, is essential for transposition. As TPASE binds to the *Ac* 5' region responsible for promoter activity, we suspected that it can also affect the activity of its own promoter. This type of autoregulation has not been shown previously for *Ac*. In order to test autoregulation of *Ac* by *Ac* TPASE, plasmids harboring a fusion of either the *Ac* promoter alone (pAcpGUS) or the promoter with the element 600-700bp untranslated leader (pAcplGUS) fused to the β -Glucuronidase (GUS) reporter gene were made. GUS activity driven from these two constructs was tested in absence or presence of TPASE which was expressed in *trans*. Such assays were carried out in two different systems: tobacco protoplasts and transgenic plants. In both system TPASE-mediated repression was detected. The first 251 bp of the element were enough to confer TPASE-mediated repression. This down regulation of *Ac* by its own TPASE might be a way to maintain low basal levels of mutagenicity.

1.2 Developmental regulation of *Ac* promoter - To test whether *Ac* promoter has different levels of activity in different tissues, GUS activity was tested in different organs of transgenic tobacco plants harboring an *Ac* promoter-GUS fusion. This work, together with part 1.3 (below) is in preparation for a publication in which BARD will be acknowledged.

In situ GUS staining of different organs of AcplGUS-GUS transgenic tobacco plants

X-gluc staining of different organs were carried out for few independent transformation events of AcplGUS transgenic tobacco plants as well as for tobacco wild type plants (negative control) and plant harboring the 35S-GUS fusion (positive control). As shown in Figure 1, GUS activity driven from the *Ac* promoter was present in all tissues, at low levels. In all tissues it was stronger in young or dividing tissues

than in old or non-dividing ones (preliminary results were also shown in the progress report). This activity was obtained only after staining tissues 3-4 times subsequently, implying again the weakness of the element promoter in contrast to the strong 35S CaMV promoter with was intensely stained. No background staining was found in the WT. (Fig. 1).

Fig 1. X-gluc staining of different organs. Different organs from AcplGUS transgenic plants, wild type tobacco plants (*Nicotiana tabacum* var. Samsun - NN), and transgenic plants carrying the 35S-GUS fusion were dissected. GUS activity was detected by hystological staining using X-gluc. X-gluc staining was repeated subsequently for each organ three times. (A) AcplGUS plants, (B) 35S-GUS plants, (C) NN plants.

Comparison of GUS activity in young and old AcplGUS plants by the fluorimetric GUS assay

As it could be argued that stronger staining in young tissues is due to the permeability of the tissues to the x-Gluc substrate, and in order to quantify GUS activity in young (dividing) vs. old (fully differentiated) tissues GUS activity was measured in young vs. old leaves of AcplGUS-GUS transgenic tobacco plants using a fluorimetric assay.

As shown in Figure 2, in all six different AcplGUS transformation events GUS activity was much higher in young leaves than in old ones. On the average, GUS activity is 30 fold higher in young tissues. The opposite ratio (0.52), i.e. higher activity in old leaves, was obtained in plants carrying the 35S CaMV promoter, indicating that this phenomenon was specific to the Ac promoter. Thus, the difference between young and old leaves is not a consequence of position effect or of tissue permeability. One possibility which was examined further is that Ac promoter is more active in dividing cells and might be cell cycle regulated.

Expression of AcplGUS fusions in tobacco cotyledons

Based on the results obtained from X-gluc staining in different organs and on GUS activity in young vs. old leaves we decided to check whether expression of GUS reporter gene from the AcplGUS fusion correlates with post-germinative cell divisions in cotyledons. Pattern of cell divisions in cotyledons are described in: Fridlender et al., 1996, 199: 307-313, an article where BARD is acknowledged. The work on cell divisions in the cotyledon was not originally planned. It was added in order to allow a better interpretation of staining patterns in the cotyledon and to further

establish the relation between cell divisions and promoter activity. This relation was tested by measuring GUS activity during days 2 to 7 after sowing. As shown in Figure 3 the same time course of GUS activity was found in two different transformation events of the *AcplGUS* fusion (#39 and #40). GUS activity two days after sowing is low. However, at the third day GUS activity reaches its highest levels. This activity is fully correlated with the time course of cell divisions in cotyledons as reported by us previously (Fridlender et al., 1996b). Following this peak GUS activity decreased until day six when it increased again (Fig. 3). It is important to notice that after day six, GUS activity disappeared in cotyledons and appeared in meristems of the first leaf (data not shown). At this points there are no cell divisions in the cotyledon but cell divisions are restricted to the meristem.

Based on the correlation between time course of cell divisions and GUS activity, and comparison of young vs. old leaves we conclude that *Ac* promoter is active in all dividing tissues. Therefore, it is possible that transcription, e.g. expression driven from the *Ac* promoter may be cell cycle regulated.

A

1.3 Cell cycle regulation of *Ac* promoter - The fact that *Ac* transposition was found to occur during DNA replication (Greenblatt and Brink, 1963) and based on our preliminary results showing that the *Ac* promoter has stronger activity in dividing tissues lead us to test whether *Ac* transcription of TPASE is also cell cycle regulated. This hypothesis was tested using a BY2 tobacco cell suspension which proliferates rapidly and whose growth can be synchronized and blocked at specific stages of the cell cycle. A series of preliminary experiments were done using cell cycle blocking agents and a cell sorter (FACS) to optimize the synchronization conditions. Hydroxyurea was found to efficiently block cells in the early S phase, and Oryzalin was used to block cells during Mitosis. BY2 cells transformed with AcpGUS or with the WT-*Ac* were prepared and treated with Hydroxyurea or Oryzalin. Cells were harvested from the following phases: Stationary (untreated), Early S (after Hydroxyurea treatment) and M (after Oryzalin treatment). RNA was extracted from each treatment and the amount of steady state RNA transcribed from *Ac* promoter was determined so far only for the AcpGUS transgenic culture using an RNase protection assay. As shown in Figure 4, GUS RNA levels, transcribed under the control of *Ac* promoter increased at early S phase and kept increasing at the M phase.

Figure 4: Cell cycle regulation of the transcription of *Ac* promoter. Total mRNA was extracted from BY2 cells from the Stationary phase (top left); from cells treated with Hydroxyurea and from cells treated with Oryzalin. Cell sorting by FACS indicated that the cell cycle blocking agents were efficient, with hydroxyurea causing a strong enrichment of cells in the S phase and and oryzalin enriching for the G2/M phase. The amount of mRNA after treatment with the blocking agents, compared to the amount of mRNA at the stationary phase was determined by RNase protection. The amount of steady state transcript driven by *Ac* promoter in the AcpGUS constructs was doubled by hydroxyurea treatment and was increased by ten-fold by Oryzalin treatment.

1.4 Promoter-hosts interactions - Organ specificity as well as cell cycle regulation are the consequences of host-element interactions. Therefore, search for host factors which bind to the *Ac* 5' was carried out. Such factors are potential regulators of *Ac* transcription. We carried out few experiments aimed to test whether a protein extract from a *Nicotiana sylvestris* tobacco cell suspension, contains any proteins which binds the *Ac* 5'. We found using electro-mobility shift assays (EMSA) a hexamer motif (GGTAAA) repeated in both ends of the element which was identified as an host factor binding site. Mutation of two nucleotides within this hexamer was sufficient to eliminate binding of the protein. A series of competition assays showed binding acts in a cooperative manner between repeated motifs. Interestingly, the same GGTAAA hexamer was reported as the binding site at the ends of the *Mutator* TE. Furthermore this motif was found to be present near the ends of several other TEs suggesting that it might be involved in transposition. In addition, the fact that the host factor binding site overlaps the TPASE binding sites and results in increased promoter activity upon deletion of the first 181 bp of *Ac* (data not shown) may imply also a role in transcription regulation. These data are summarized in an enclosed Article where BARD is acknowledged (Levy et al. MGG, 1996, 251:436-441) .

2. Regulation of *Mu* promoter

The work on the regulation of Mutator promoter has been accepted for publication in the following article:

P. Joanin, R. J. Hershberger, M.-I. Benito, and V. Walbot. Sense and antisense transcripts of the maize MuDr regulatory transposon localized by in situ hybridization. Plant Molecular Biology, In Press

The Joanin et al. manuscript provides data on the following points:

MuDR encodes two genes, convergently transcribed from near-identical promoters in the terminal inverted repeats. *mudrA*, based on similarities of protein sequence, is proposed to be the transposase; by virtue of alternative splicing and 2 distinct 5' start sites, four distinct proteins of 80 - 105 kDa could be produced. Fully spliced transcript of *mudrB* encodes a 23 kDa protein of unknown function; again alternative splicing could result in 4 distinct proteins from this gene.

In addition to the complexity of splicing, substantial antisense RNA is produced from MuDR. The antisense RNA may have a major role in regulating the developmental specificity of Mutator.

In the ms. we show that

1. *mudrA* and B transcripts exist in the same cells and are nearly ubiquitous in the plant
2. antisense A is also abundant, but antisense B is a rare transcript

3. production of sense and antisense transcripts occurs only in active Mutator plants; all transcripts drop in concentration in plants that are losing activity and are missing in inactive Mutator plants
4. in developing ears of 4 different Mutator lines (with 1 or many copies of MuDR) we see the same pattern of transcript accumulation; there is a very high level in the endothelium (tapetum surrounding the embryo sac).
5. in contrast, in the developing embryos, each line has a distinctive pattern of transcript abundance. We interpret this as evidence of "position effects" on one of a few MuDRs that are actively transcribed in the soma

Cooperation and evaluation of the project

The Research supported by this BARD proposal has led to a number of important findings concerning the regulation of *Ac* and *MuDR* which were summerized in four enclosed publications. In Israel, *Ac* transcription was shown to be autoregulated; cell cycle regulated, being active in dividing tissues, in the S through M phases; and host factors were identified as putative regulators of *Ac* transposition. In The USA, *MuDR*, was shown to carry two promoters at its extremities, convergently transcribing two genes, and that alternative splicing and antisense were involved in the element regulation. These complex modes of regulation for both elements have led us to go deeper than expected into transcriptional and post-transcriptional regulation of the element, and we did not reach the part which was supposed to address the developmental regulation of *MuDR* transposition, in particular in heterologous system. This part was supposed to be carried out in Israel following the characterization done in Stanford. Some transient experiments, done in Israel with *MuDR* constructs provided by the Stanford Lab, using tobacco protoplasts, have confirmed that *MuDR* carries two converging promoters. These experiments were described in the Second year annual report but were not published or carried further as more detailed and more meaningful experiments were done in situ with transgenic plants as reported by Joanin et al. In this respect, our collaboration did involve meetings, exchange of material and of idea but did not lead to co-authorship of publications as we did not reach the stage of joint experiments with joint material.

List of publications resulting from the research project

Fridlender, M., Harrison, K., Jones, J.D.G. and A.A. Levy 1996. Repression of the *Ac*-transposase gene promoter by *Ac* transposase. *Plant J.* 9:911-917

Fridlender, M., Lev-Yadun, S., Baburek, I., Angelis, K. and A.A. Levy 1996. Cell divisions in cotyledons after germination: localization, time course and utilization for a mutagenesis assay. *Planta.* 199: 307-313

Levy, A.A., Fridlender, M., Hanania, U., Rubin, E. and Y. Sitrit 1996. Binding of *Nicotiana* nuclear protein to the subterminal region of the *Ac* transposable element. *Mol. Gen. Genet.* 251:436-441

Fridlender, M., Sitrit, Y. and A.A. Levy 1996. *Ac* promoter activity is enhanced in dividing tissues and between the S through M phases. In preparation

P. Joanin, R. J. Hershberger, M.-I. Benito, and V. Walbot. Sense and antisense transcripts of the maize MuDr regulatory transposon localized by in situ hybridization. *Plant Molecular Biology*, In Press

Patents

None.