Appendix A

Exogenous matrix binding assays with various putative MARs

Introduction

Matrix attachment regions of DNA are defined operationally by their affinity for the nuclear matrix. Identification of a matrix attachment region is accomplished by an in vitro assay of the ability of a particular sequence to bind to the nuclear matrix. In the binding assay, isolated nuclei are treated with LIS to remove the soluble proteins, predominately the histones. The non-MAR DNA is removed by restriction enzymes and we call the remaining structure the nuclear matrix. In the exogenous binding assay, DNA fragments of interest are end labeled and are incubated with the nuclear matrix. Endogenous nuclear DNA serves as a competitor. After incubation with labeled DNA, the insoluble nuclear matrices with bound DNA are separated from non-MAR DNA by centrifugation and digested with protease. The DNA fragments from the non-MAR (supernatant) and MAR-containing (pellet) fractions are separated by electrophoresis on agarose gels and visualized by autoradiography. If the particular DNA fragment of interest is present in the bound fraction, then it is considered to have MAR binding activity. Labeled vector DNA present in the binding reaction serves as an internal negative control.

During the course of studies that produced this dissertation, matrices used for protein analysis and DNA protein blotting experiments were often tested for competency in the exogenous binding assay. These quality control assays confirmed that the matrices used for protein studies correspond to the matrices shown to interact with MAR DNA (Hall et al., 1991; Michalowski et al., 1999). In addition to testing the matrices for ability to bind to the well characterized MARs from tobacco, occasionally putative MARs from other labs were assayed for matrix binding activity. Results of these exogenous binding assays are presented here.

Finally, we present results from an abbreviated attempt to produces a MAR fragment small enough to be used in electrophoretic mobility gel shift assay. These experiments were initiated with the assistance of Nancy Gillikin, who left our lab before the experiments could be completed. These experiments demonstrate that a 650bp subfragment of the S116-1.1B MAR described previously (Michalowski et al., 1999) retains strong matrix binding activity. The subfragment was cloned and is available for future study. Results of exogenous binding experiments with this, the S116-1.1B PacI fragment, are also presented in this section.

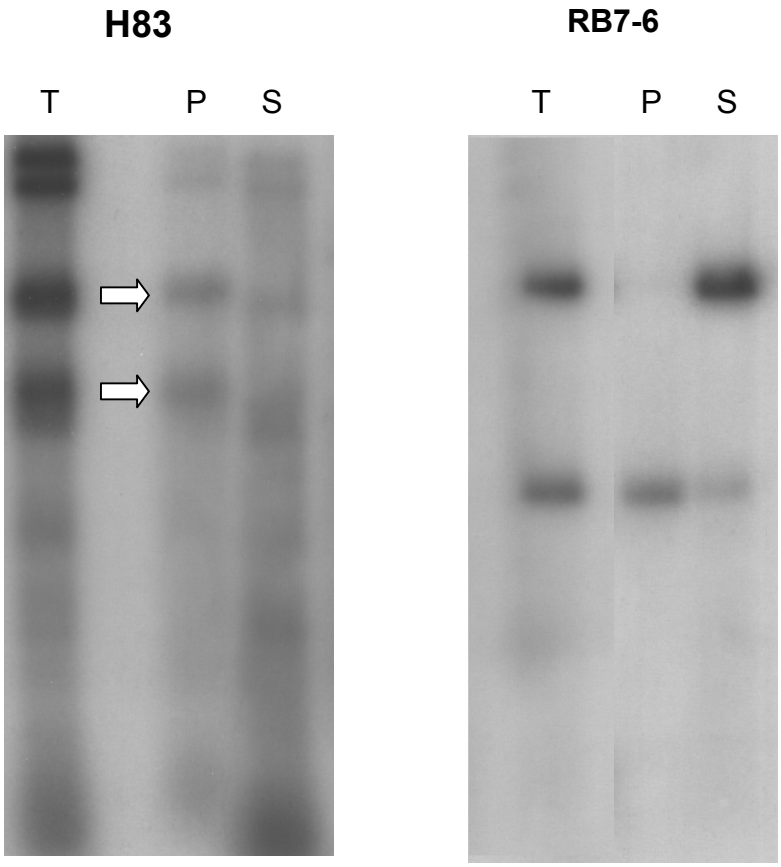

Figure A1. Exogenous binding assay s using "H83" putative MAR from Antonius Matzke. Matrices were prepared with XbaI and HindIII restriction enzymes. The putative MAR, which is included on both 1.9 and 2.9 kb fragments (marked with arrows), does bind to matrices, at nearly the same level as the RB7-6 MAR.
(This data was published in Plant Cell, 9: 1251-1264.)

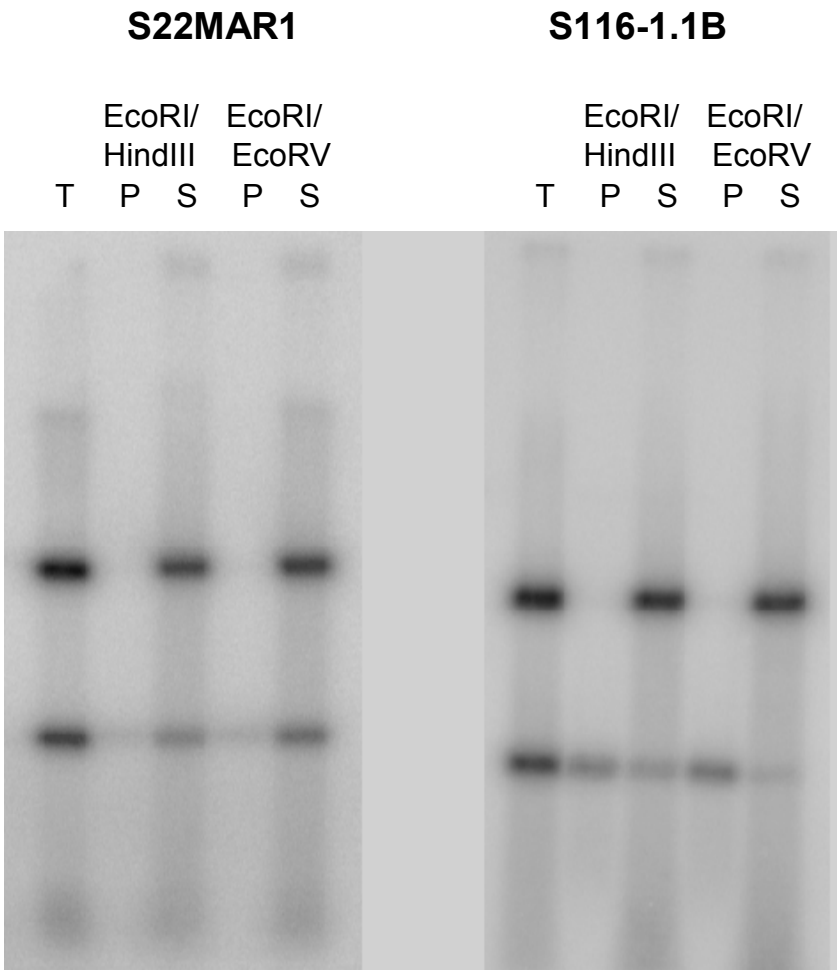

Figure A2. Exogenous binding assay s using "SSMAR1" putative MAR from Marjori Matzke. Matrices were prepared with either EcoRI and HindIII restriction enzymes, or with EcoRI and EcoRV restriction enzymes. The putative MAR does bind to matrices, but not as well as the strong MAR S116-1.1B.

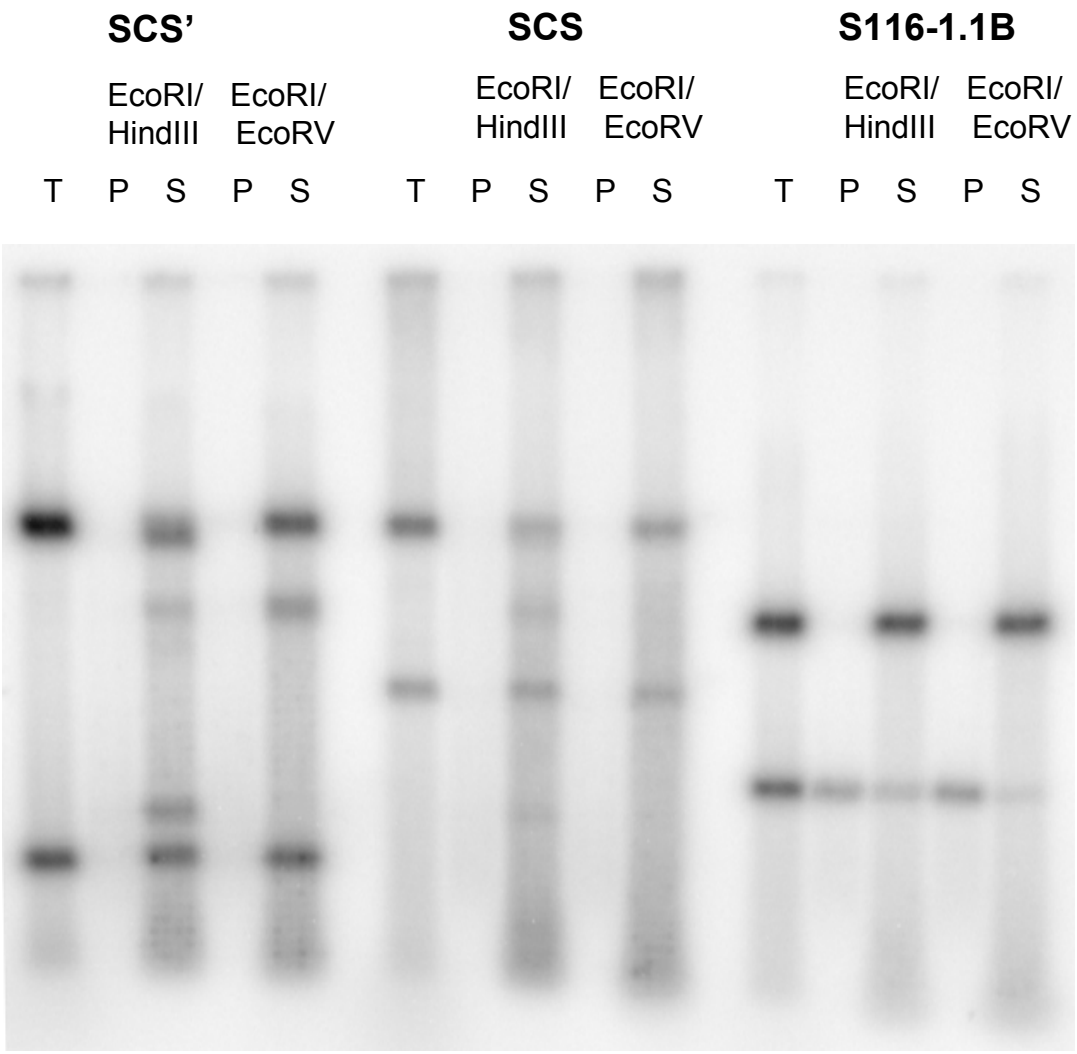

Figure A3. Exogenous binding assay s using SCS and SCS' chromatin elements. Matrices were prepared with either EcoRI and HindIII restriction enzymes, or with EcoRI and EcoRV restriction enzymes. The SCS' element (0.5kb), and the SCS element (1.8 kb) do not bind to matrix preparations in the conditions used in these assays.

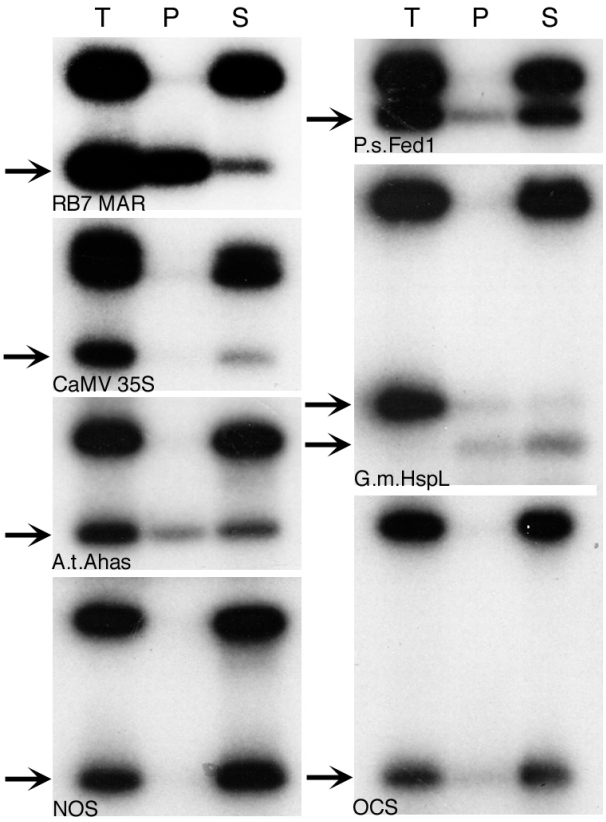

Figure A4. Exogenous binding assay s using various promoters. Matrices were prepared with either XbaI and HindIII restriction enzymes. The AtAhas, GmHspL, OCS, and PsFed1 promoters all bind to tobacco matrices; however, the 35S and NOS promoters did not bind strongly enough to be detected. None of the promoters bind as well as the strong RB7-6 MAR.

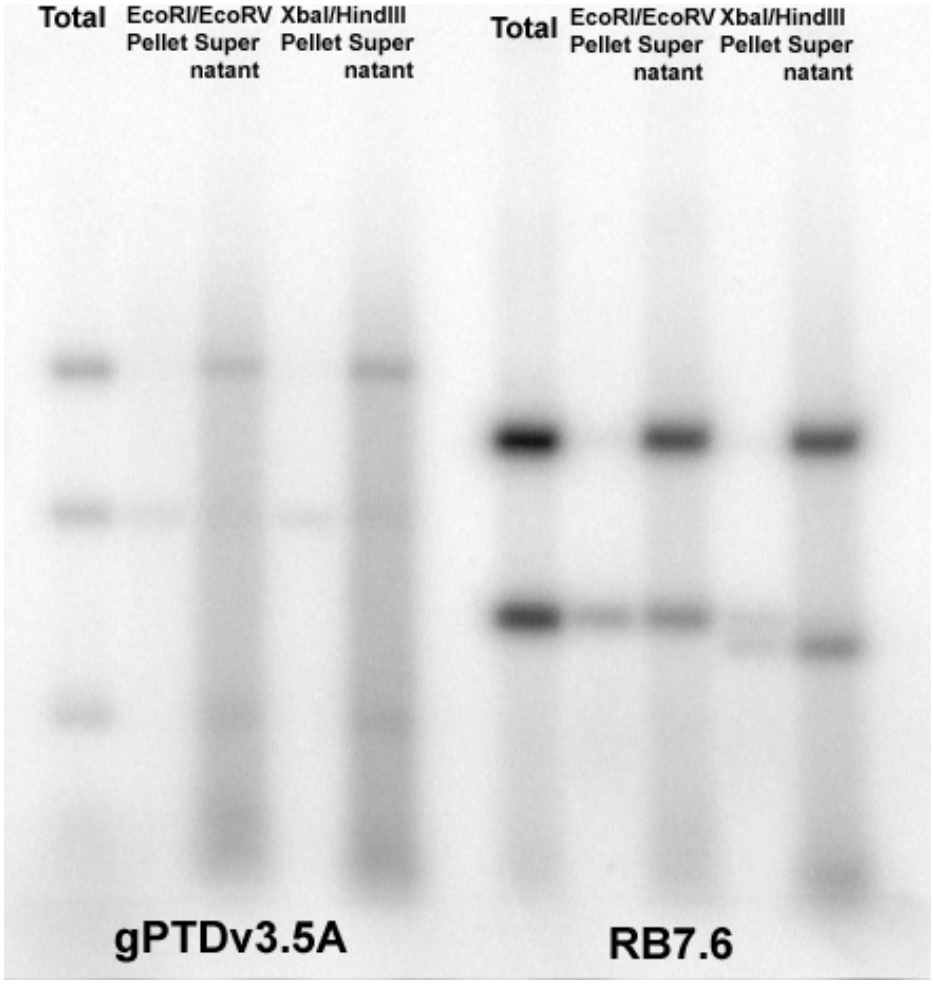

Figure A5. Exogenous binding assay of the putative poplar (Poplar trichocarpa) MAR, 1.9 kb. Matrices were prepared with either EcoRI and HindIII restriction enzymes, or with EcoRI and EcoRV restriction enzymes. This MAR binds weakly, relative to the strong RB7-6 MAR, but more strongly than the 4 kb gPTDv3.5A vector/genomic poplar DNA band or the 0.6 kb poplar sequence band.

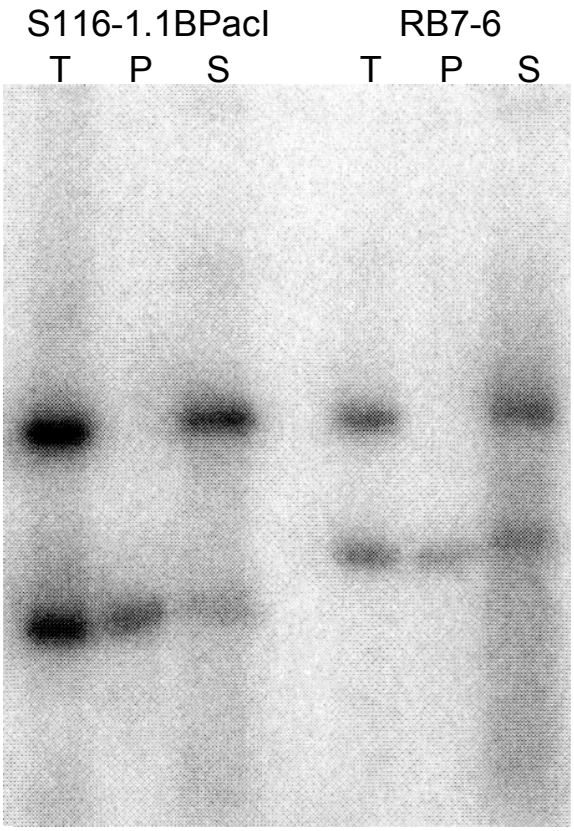

Figure A6. Exogenous binding assay of the S116-1.1BPacI fragment. The S116-1.1B MAR was cut with PacI and the 660 bp fragment was cloned into the pNEB193 PacI site. LIS extracted matrices were prepared using XbaI and HindIII restriction enzymes. This S116-1.1B fragment binds with affinity similar to the strong RB7.6 MAR.