

## Chapter 3

### Identification of Plant MAR Binding Proteins

#### Introduction

Our understanding of the nature of the plant nuclear matrix is incomplete. While there has been a great deal of interest in the interactions between MAR DNA and the nuclear matrix, the study of these interactions has been difficult. The nuclear matrix is by definition an insoluble structure, making the study of individual components quite challenging. Individual protein components from the matrix can be separated only by use of strong protein denaturants. Denaturation of individual proteins results in loss of higher order structure and usually causes loss of native function. Correct protein folding often requires specific interactions, which may not be achieved after separation of the protein from its native surroundings. In addition, since MAR DNA is typically large and non-homogeneous many of the classical methods for the identification of DNA ligands are not practicable. Most methods for the study of DNA protein interactions require small, well defined DNA sequences. These methods include electrophoretic mobility shift assays, chromatographic separation, coprecipitation of tagged DNA protein complexes, and expression library screening. However, most MAR fragments isolated have a size of approximately 1 kb and have no consensus sequence (Michalowski et al., 1999). Use of the above techniques

would be expected to identify numerous general DNA binding proteins including histones and HMG proteins, as well as specific MAR binding proteins.

One of the biochemical tools available for study of DNA binding proteins is the DNA protein blot binding assay, also known as a southwestern blot (Miskimins et al., 1985). This technique allows us to circumvent the two most formidable difficulties in identification of MAR binding proteins. Proteins can be denatured and separated by size, and a large MAR DNA probe can be used.

For this assay, proteins are denatured and separated electrophoretically. The separated proteins are transferred to a membrane, and the membrane is probed with labeled DNA. By optimizing binding conditions and levels of competitor DNA, proteins with specific DNA binding function can be identified. This method allows for the use of larger probes than those used with many other techniques. Of course, since the proteins bound to the membrane have endured harsh conditions during their isolation and separation, only those proteins that retain or regain their native function can be identified with this assay.

The DNA protein blot technique has been used to identify MAR binding proteins. The chicken protein ARBP was the first MAR binding protein to be reported, and was identified using this assay (von Kries et al., 1991). Subsequently, several other nuclear proteins have been identified as MAR binding proteins by this assay (Luderus et al., 1992; Hatton and Gray, 1999; Romig et al., 1992; von Kries et al., 1994; Rzepecki et al., 1995a and 1995b).

To identify MAR binding proteins in wheat and tobacco, the DNA protein blot method was employed. Several candidate proteins were identified in both plant species. One of the proteins identified is histone H1, a general DNA binding protein. This protein was identified by electrophoretic mobility and by immunological similarity to wheat H1. The nuclear intermediate filament proteins lamin A, B and C have previously been reported as MAR binding proteins in humans (Luderus et al., 1992, 1994). However, lamins have not yet been demonstrated to be present or to bind MAR DNA in plants. Therefore, immunoblot analysis was conducted to determine whether the MAR binding proteins seen in this work might be related to lamins. These results suggest that an intermediate filament-like protein is present in tobacco matrices, but this protein does not interact with DNA in the DNA protein blot assay.

Using the DNA protein blot assay, three tobacco nuclear matrix protein bands were identified as strong, specific MAR binding proteins. These proteins have apparent molecular weights near 70 kDa on SDS acrylamide gels. These same protein bands are present in matrices prepared by both the LIS method and by the NaCl method. The protein migrating fastest on SDS acrylamide gels was used to obtain protein sequence. The sequence information was used to clone the genes encoding a family of related proteins from the model plant *Arabidopsis thaliana*. Results of the cloning are presented in the next chapter of this work.

Additionally, another of these proteins may have homology to a recently identified wheat MAR binding protein AHM1 (Morisawa et al., 2000). AHM1 has been cloned

and shown to contain an AT-hook, a DNA binding domain found in HMG proteins, a class of low molecular weight chromosomal proteins. Immunoblotting assay results presented here suggest that HMG proteins are not present in the nuclear matrix, but that one of the MAR binding proteins identified by the DNA protein blot does have antigenic similarity to HMGa.

## **Materials and Methods**

### *Electroblotting of protein to PVDF membranes*

Polyacrylamide gel separated proteins were transferred to Immobilon PVDF (Millipore) membranes according to manufacturers instructions. Gels were electroblotted using a semi-dry blotting apparatus (BioRad) at constant current of 0.8 mA/cm<sup>2</sup> for one to two hours to membranes prewet in methanol and equilibrated in transfer buffer. After transfer, proteins were fixed to the membrane by drying the membrane completely at room temperature. To determine the position of proteins on the membranes, some membranes were stained for one minute in 0.1% Coomassie brilliant blue (R-250) in 50% methanol, 1% acetic acid, and destained in several changes of 50% methanol.

### *Probe labeling*

Radioactively probes were end labeled by Klenow fill-in reaction (Sambrook et al., 1989). Probes were separated from unincorporated nucleotides by Sephadex G-50 spin column purification. Radioactively labeled probe mixtures contained both labeled vector and MAR insert DNA. Non-radioactively labeled DNA probes were end labeled using digoxigenin-conjugated to dUTP or biotin-conjugated to dATP. Labeled dNTPs were added to the 5' ends of gel isolated MAR DNA fragments using terminal transferase, according to manufacturers instructions (Roche/Boehringer Mannheim). Unincorporated nucleotides were removed by ethanol precipitation.

### *DNA protein blotting*

After electroblotting, membranes were rewet and then blocked for at least one hour in binding buffer (10 mM Tris-HCl, 50 mM NaCl, 2 mM EDTA, pH 7.5) supplemented

with 5% (w/v) nonfat dry milk. Membranes were rinsed in binding buffer plus 0.5% (w/v) nonfat dry milk. For the standard DNA binding assay, membranes were incubated at room temperature in 2 ml binding buffer with 25ng end labeled probe and 5 µg unlabeled restriction digested E. coli DNA. E. coli DNA was obtained from Sigma, and digested with EcoRI, phenol/chloroform extracted, and resuspended in 10mM Tris-HCl/EDTA, pH 7.5. Incubations were conducted for 90 minutes to overnight, with gentle agitation. Membranes were washed 4 times in binding buffer plus 0.5% (w/v) nonfat dry milk.

#### *Immunoblotting*

After electroblotting, membranes were rewet and then blocked for at least one hour in binding buffer (20 mM Tris-HCl, 0.5M NaCl, pH 7.5) supplemented with 3% BSA. Membranes were rinsed briefly in binding buffer, then incubated with primary antibody in binding buffer supplemented with 1% BSA. Membranes were incubated 1 hour with gentle agitation at room temperature. Membranes were washed with vigorous agitation 3 times in 100 ml washing buffer (binding buffer plus 0.5% Tween-20) for 15 minutes per wash. Incubation in secondary antibodies conjugated to alkaline phosphatase proceeded identically to conditions for primary antibody.

#### *Detection of probe*

After incubation and washes, probes were detected by standard methods. Membranes probed with radiolabeled probes were exposed directly to film or placed in phosphoimager cassettes. Alkaline phosphatase conjugated secondary antibodies were detected using CDP-Star chemiluminescent detection system (Roche/Boehringer Mannheim). After hybridizations and washes, membranes were washed briefly in MA buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5) plus 0.3% Tween 20. One ml CDP-

Star reagent per 100 cm<sup>2</sup> membrane (0.25mM final concentration), diluted in MA buffer, was added to each membrane and incubated in hybridization bags for 5 minutes at room temperature. Excess CDP-Star reagent was wicked away from membranes with paper towels. Membranes were wrapped in fresh hybridization bags, and exposed to film.

## **Results**

### *DNA protein blot experiments*

DNA protein blot experiments were conducted using well characterized tobacco MAR fragments as probes. To confirm that the proteins identified with this probe were not specific to a particular MAR, DNA protein blots were conducted using both the tobacco RB7-6 MAR and the tobacco S116-1.1B MAR. The primary MAR probe used in these experiments was the tobacco RB7-6 MAR fragment. Both MARs have been characterized as having strong affinity to the nuclear matrix, based on the endogenous matrix binding assay (Michalowski et al., 1999).

Figures 3-1 through 3-4 present results of these DNA protein blot experiments using tissues from wheat or tobacco, and a comparison of the proteins detected in matrices prepared using each of the most common matrix preparation protocols. In Figure 3-1 the RB7 MAR DNA was used in a DNA protein blot experiment to probe histones and nuclear matrix proteins from wheat germ nuclei. In the first lane of this blot are wheat germ histone proteins. These proteins bind to the labeled MAR DNA. However, since these histone proteins are known to be general DNA binding proteins, this association is probably not specific. In lane two of this blot, numerous proteins bind to the MAR DNA probe. As many as ten protein bands can be visualized by association with the labeled MAR DNA probe, not counting the lower molecular mass proteins that correspond in size to the histones in lane one. The proteins seen as DNA binding proteins in this assay most likely represent a mixture of specific MAR binding proteins and less specific DNA binding proteins. These DNA protein blots were

conducted in the presence of 100 fold non-specific DNA competitor, which should reduce or eliminate non-specific interactions. However, further experiments to determine the specificity of the higher molecular weight proteins that bound DNA in this experiment were not conducted.

In figure 3-2, DNA protein blot analysis of tobacco culture cell nuclear matrices reveals three bands migrating near 70 kDa molecular weight (lane 1). The specificity of these results was demonstrated by probing nuclear matrix protein from the same blot with a non-MAR DNA: the vector pBluescript II. This probe was linearized and end labeled by the same protocol used to label the MAR probe. The binding reaction contain end-labeled vector DNA as well as labeled MAR DNA. To identify bands specifically bound by the MAR DNA, and not by non-MAR DNA, additional experiments were conducted. DNA protein blot analysis with end labeled vector was chosen as a negative control since the vector has been shown not to bind to matrices in the exogenous binding assay. As can be seen in column two of Figure 3-2, only the low molecular weight proteins bind to the non-MAR DNA. Additional DNA protein blot experiments using non-radioactively labeled gel-isolated MAR DNA fragments also confirm that the bands migrating near 70 kDa are binding to MAR DNA.

The binding proteins seen in the DNA protein blot using two different MARs were compared, and the results are shown in Figure 3-3. Two adjacent strips from the same blot of tobacco nuclear matrix proteins were probed separately under identical conditions, using either the tobacco RB7 MAR or the tobacco S116-1.1B MAR. In this experiment, the same three bands at approximately 70 kDa molecular weight are

recognized by each MAR. It is also apparent in these results that the protein migrating at the highest molecular weight binds less probe than the two lower molecular weight bands. This weaker binding was seen in other DNA protein blot experiments. In some cases this band is not observed at all. For an example, see Figure 3-4.

In chapter 2, comparison of the SDS acrylamide gel separated proteins from tobacco matrices prepared by either the LIS protocol or by the NaCl protocol demonstrated that the protein profiles are quite similar. To demonstrate that the 70 kDa MAR binding proteins seen in tobacco matrices prepared by the LIS method are also present in NaCl matrices, a DNA protein blot analysis of matrix proteins from NaCl matrices was done. As seen in Figure 3-4, the tobacco MAR binding proteins seen in LIS matrices appear to be the same as the MAR binding proteins seen in NaCl matrices. As mentioned above, the weaker MAR DNA binding protein seen in some DNA protein blots is not visible in this result. This “weaker” binding may be due to the relative abundance of the other proteins compared to the “weak” band. As discussed in Chapter 2, and shown in Figure 2-4, the highest molecular weight band of the group of three ~70 kDa MAR binding proteins is less abundant in NaCl matrices, relative to LIS matrices. In most nuclear matrix preparations using either method, the highest molecular weight band is less abundant than the other two bands, based on relative Coomassie Blue staining intensities.

#### *Immunoblotting of matrix protein with IFA*

Of the MAR binding proteins identified as of the date of these initial DNA protein blotting results, only the lamins were reported to have a similar molecular weight to

the tobacco proteins seen on these blots. Lamins are a member of the intermediate filament family of proteins. To ascertain whether these tobacco proteins might be plant lamins, or related proteins, the proteins were subjected to immunoblot analysis. For these experiments, the mouse antibody IFA (ATCC number TIB131) was employed. IFA is a monoclonal antibody that recognizes a conserved epitope present in all known intermediate filament proteins (Pruss et al., 1981). Tobacco nuclear matrix proteins were separated on SDS polyacrylamide gels and transferred to PVDF membrane. Strips from the membrane were probed with the IFA antibody, and the proteins recognized by this antibody were subsequently detected. Figure 3-5 presents the results of this analysis. IFA did not recognize the MAR binding proteins seen in the DNA protein blot experiments. Instead, it most strongly recognized a protein migrating at approximately 52 kDa. A band at approximately 32 kDa is also recognized by the IFA antibody. The membrane strip used in this experiment is taken from the same blot used for figure 3-3. Comparison of the bands from these two results clearly indicates no overlap between the protein bands recognized.

#### *Immunoblotting of matrix protein with anti-H1 antibody*

As can be seen in Figure 3-2, lower molecular weight protein bands recognize both MAR DNA and non-MAR DNA probes in the DNA protein blot assay. We suspected that these lower molecular weight bands represented histone proteins. The largest tobacco histone protein (H1) has a reported molecular weight of approximately 30 kDa (Szekeres et al., 1995). Since there is a large discrepancy between the electrophoretic mobility seen in the DNA protein blots and the reported molecular weight of histone H1, immunoblotting analysis with a polyclonal antibody to wheat

H1 was performed. As seen in Figure 3-6, the protein band recognized by wheat anti-H1 corresponds in size to the non-specific DNA binding protein seen in DNA protein blots of tobacco nuclear matrices.

*Immunoblotting of matrix protein with anti-HMGa antibody*

The High Mobility Group (HMG) proteins are a group of low molecular weight chromosomal proteins. These proteins were initially discovered as a contaminant of histone protein preparations. One theory of chromatin regulation suggests that the linker histone H1 is competed by HMG I/Y type proteins to promote chromatin conformations that are more accessible to transcription factors (Zhao et al., 1993). Since H1 is found in the nuclear matrix, we wished to test whether the putative H1 agonist was also present. To test whether HMG I/Y type proteins associate with the nuclear matrix, immunoblot analysis of tobacco nuclear protein was conducted. (Note: These experiments were done in collaboration with Jennifer L. Ingram.)

The results of these experiments are presented in Figure 3-7. In order to resolve the lower molecular weight HMG proteins, the nuclear proteins were separated by electrophoresis through 15% polyacrylamide/SDS gels. Figure 3-7 presents data for total nuclear protein, histone protein and nuclear matrix protein. Nuclear matrices were prepared using the LIS protocol. In part 3-7A of this figure is a replicate Coomassie stained gel of the proteins used in parts 3-7B and 3-7C. Figure 3-7B presents results of the immunoblot analysis of these nuclear proteins. This blot was probed with a polyclonal antibody to wheat HMGa, an HMG I/Y type protein (Spiker and Everett, 1987). As expected, the antibody recognizes a low molecular weight

tobacco protein in the total nuclear fraction in lane 1. This probably is the tobacco homolog of wheat HMGa. In the tobacco histone fraction (Figure 3-7B, lane 2), no bands are recognized by the wheat HMGa antibody. This correlates with results seen in similar immunoblotting experiments (JL Ingram, personal communication).

Tobacco HMG proteins in tobacco histone preparations are not normally detected by the wheat HMGa antibodies. In Figure 3-7A, lane 3 a higher molecular weight protein was recognized by the wheat HMGa antibody. This protein migrates at approximately 75 kDa, a much higher molecular weight than any HMG protein. Interestingly, this size is very close to the size of the tobacco MAR binding proteins that were detected in the DNA protein blot experiments. Figure 3-7C

presents a DNA protein blot of proteins run and transferred to the same blot used in Figure 3-7B. The 70 kDa proteins seen in previous blots from lower percentage gels are not resolved well here, and appear as a single band. Alignment of the bands recognized by the probes in each of these results suggests that the protein recognized by wheat anti-HMGa is the same molecular weight as at least one of the proteins that bound to MAR DNA. The second, lower molecular weight band seen in the matrices lane is probably histone H1. Interestingly, a corresponding band of this size is not seen in the histone lane of this DNA protein blot.

We would also have expected the 75 kDa band recognized by HMGa and the MAR DNA to be recognized by both probes in lane 1, total nuclear protein. The DNA protein blot recognized protein of this approximate molecular weight, but the HMGa antibody did not generate a signal. Additional experiments have demonstrated that the HMGa antibody does recognize a 75 kDa protein in nuclear extracts (JL Ingram

personal communication). The anomalous result seen here may be a reflection of differences in the amount of the 75 kDa protein transferred to the membrane in this particular experiment. Additional questions about the amount of protein transferred are raised by the fact that the DNA protein blot detects the H1 histone in the nuclear matrices, but not in total histone preparation. However, these anomalies do not affect our conclusions regarding the presence of HMG I/Y type proteins in the nuclear matrix.

## **Discussion**

DNA protein blot experiments were conducted using end labeled tobacco MAR DNA as probe. The goal of these experiments was to identify nuclear matrix proteins with specific affinity for MAR DNA. The primary MAR probe used in these experiments was the well characterized tobacco RB7-6 MAR fragment. This fragment was originally isolated from the region 3' of the root specific RB7 gene (Conkling et al., 1990). This 1.1 kb fragment has been characterized extensively as a strong MAR (Hall et al., 1991; Michalowski et al., 1999). It has also been used extensively in transgenic experiments, where it has been shown to increase expression levels when used to flank transgenic constructs in both plant cells (Allen et al, 1996) and in whole plants (Ülker et al., 1999). Due to the interesting features of this tobacco MAR DNA sequence, it has been used as the primary MAR probe in the DNA protein blot experiments.

A second probe has also been utilized in these experiments. The S116-1.1B MAR was isolated by a shotgun cloning approach which selected DNA fragments based on their association with the nuclear matrix. Based on the endogenous nuclear matrix binding assay, described in chapter 2, this 1.0 kb fragment is also a strong MAR (Michalowski et al., 1999).

MAR binding proteins were identified in matrices prepared from both wheat and tobacco matrices using the DNA protein blot technique, as seen in Figure 3-1. A larger number of candidate MAR binding proteins were isolated from wheat than from

tobacco. The reason for this difference is not clear. This could reflect an increased stability of the wheat proteins relative to tobacco proteins during the matrix isolation. They could also be proteins that do not have homologs expressed in the tobacco tissue used in these experiments. Alternatively, there may be proteins artifactually recovered from the wheat germ tissue used in this study that are not recovered from tobacco cultured cells, due to differences in the nuclear isolation protocols. However, rather than focus energy on these observed differences, we chose instead to pursue identification of the MAR binding proteins from tobacco. In tobacco, three protein bands, ranging in electrophoretic mobility from approximately 68 kDa to 75 kDa, were detected (Figure 3-2). These proteins were shown to bind strongly to MAR DNA probes, but not to a non-MAR. The tobacco proteins identified as MAR binding proteins in this work bind to both of the MARs tested, as shown in Figure 3-3. This result supports the idea that these proteins are general MAR binding proteins.

The size and MAR binding character of the tobacco proteins identified in the DNA protein blot experiments were highly similar to lamin proteins identified in animal cells (Luderus et al., 1992, 1994). These facts suggested that the MAR binding proteins could be plant homologs of animal lamins, a member of the intermediate filament family of proteins. Immunological evidence has suggested that lamin-like proteins are present in plant nuclei and plant nuclear matrices (McNulty and Saunders, 1992; Minguez, 1993; Frederick et al., 1992). However, no plant lamins have yet been identified as MAR binding proteins or cloned from plants. To determine whether the MAR binding proteins identified in this work might be lamin-like proteins, we used an antibody reported to recognize an epitope common to all tested intermediate filament

proteins. The antibody used is IFA (intermediate filament antigen) a monoclonal antibody generated from mouse (Pruss et al., 1981). This antibody had been previously been used extensively in animal studies, and has been used to identify intermediate filaments (Dawson et al, 1985) and lamin like proteins (McNulty and Saunders, 1992; Minguéz, 1993; Frederick et al., 1992) in plants.

Figure 3-5 presents results from the immunoblotting of tobacco nuclear matrix protein with IFA antibody. The three approximately 70 kDa proteins identified as MAR binding proteins do not react with the IFA antibody. Instead, two proteins at approximately 32 kDa and 52 kDa do react. It is not clear how closely related these proteins may be with intermediate filaments. They probably represent proteins with enough homology to the IFA epitope to be recognized under the conditions used in these experiments. They have higher electrophoretic mobility than the plant proteins identified as lamin-like in previous studies (Beven et al., 1991; McNulty and Saunders, 1992; Minguéz, 1993; Frederick et al., 1992).

To confirm our suspicion that the low molecular weight DNA binding proteins seen in the DNA protein blots represented histones, we probed blots of SDS acrylamide gel separated proteins with an H1 antibody. The H1 antibody used is a polyclonal antibody generated against wheat H1 histone. A side by side comparison of adjacent strips of membranes blotted with matrix protein demonstrates that the non-specific DNA binding protein seen in Figure 3-2 is histone H1. The preparation of nuclear matrices removes the majority of histone proteins, but some histone protein remains

associated with the matrix. It is not clear whether the association is biologically relevant.

It has been suggested that MARs are sites of nucleation for histone H1 assembly (Izzuaralde et al., 1989; Käs et al., 1993; Zhao et al., 1993). In this model, MARs form the base of loops that constitute units of differentially compacted chromatin. MAR DNA can be the nucleation point for H1 mediated compaction of chromatin. Alternatively, as has been shown in vitro with animal proteins on a linearized template (Zhao et al., 1993) HMG of the I/Y class can selectively titrate H1 away from MAR DNA to allow transcription of a reporter gene. Similar results using maize proteins support the extension of the hypothesis to plants (Zhao and Grafi, 2000), where HMG proteins have significant differences from their animal homologs (Grasser, 1995). Thus, it is hypothesized that the MARs are a target for regulation of DNA compaction by the antagonistic interaction of HMG I/Y type proteins with histone H1.

To investigate whether this interaction might be mediated by association with the nuclear matrix, immunoblotting analysis was conducted. Polyclonal antibodies to wheat HMGa, an HMG I/Y like protein, were used to probe nuclear proteins that had been electrophoretically separated and transferred to PVDF membranes. The immunoblot analysis detects a band in tobacco nuclear protein, which migrates at the expected molecular weight of HMGa. The antibody did not detect the same band in the tobacco nuclear matrix. Interestingly, a larger protein of approximately 75 kDa was detected in the tobacco nuclear matrix. The identity of this protein has not been determined. The 75 kDa band may represent the tobacco homolog to the wheat

protein AHM1. AHM1 has recently been reported as a MAR binding nuclear matrix protein containing the “AT-hook” DNA binding domain present in HMG I/Y type proteins (Morisawa et al., 2000). In conclusion, this data does not support involvement of the tobacco nuclear matrix with an interaction between histone H1 and the plant homolog of HMG I/Y.

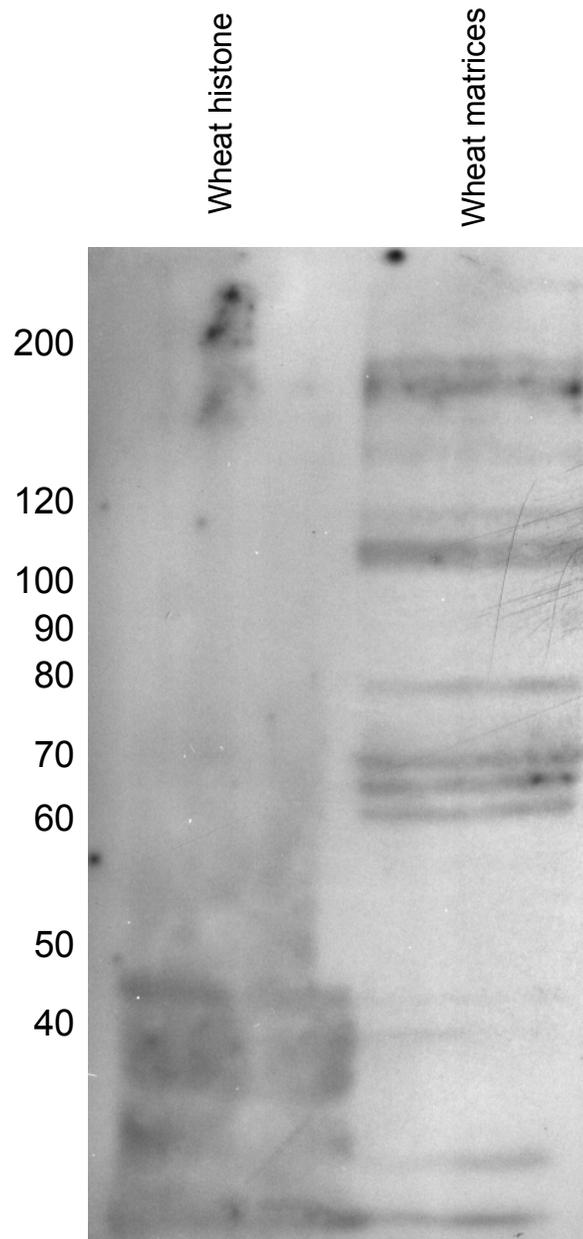


Figure 3-1 . Identification of MAR binding proteins from wheat nuclear matrices. Wheat germ histone and matrix proteins were separated by electrophoresis on an SDS acrylamide (8%) gel, transferred to PVDF membrane, and probed with digoxigenin labeled RB7 MAR. Three protein bands between 60-70 kDa, as well as several other proteins of higher apparent MW, bind to the MAR probe.

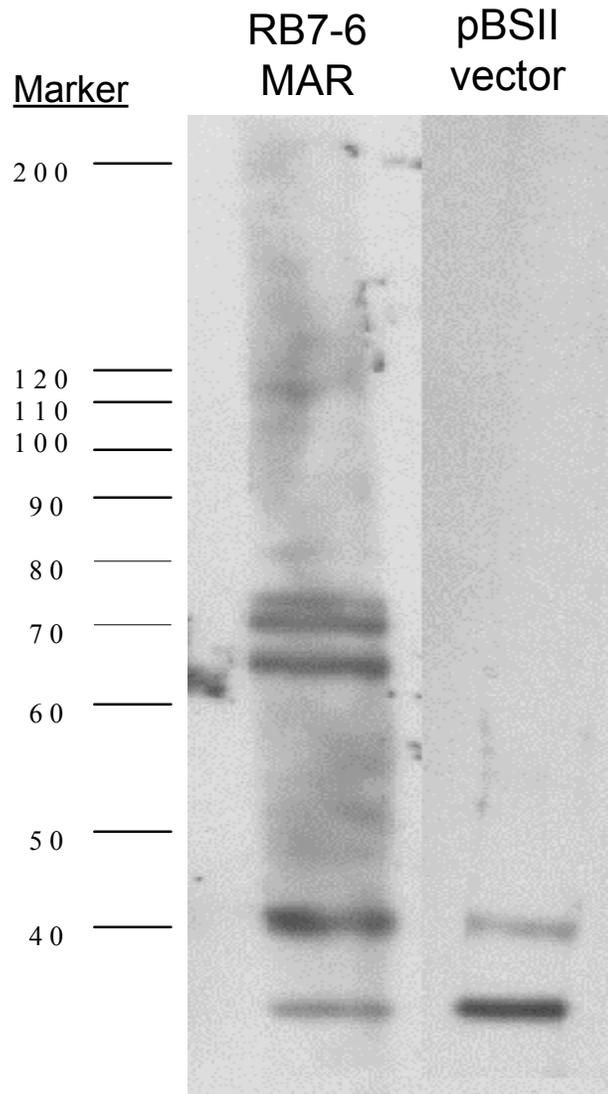


Figure 3-2. Identification of MAR binding proteins from tobacco matrices. Tobacco matrix proteins were separated by electrophoresis on an SDS acrylamide (8%) gel, transferred to PVDF membrane, and probed with  $^{32}\text{P}$  labeled RB7- 6 MAR DNA. Three protein bands between 65-75 kDa, as well as several other proteins of lower apparent MW, bind to the MAR probe. As a control, the same proteins were probed with a non-MAR DNA. Only the lower MW proteins bind the non-MAR DNA.

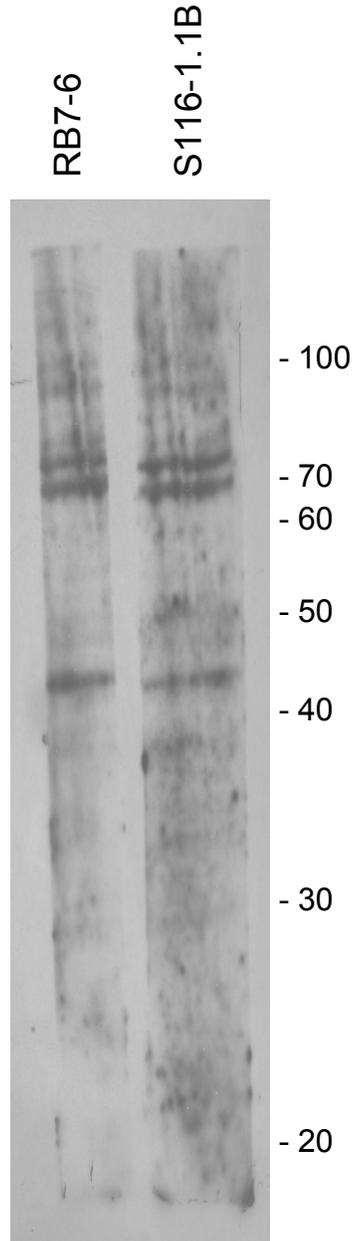


Figure 3-3. Two different tobacco MARs bind to the same proteins. Tobacco matrix proteins were separated by electrophoresis on an SDS acrylamide (10%) gel, transferred to PVDF membrane, and probed with  $^{32}\text{P}$  labeled MAR DNA fragments. Two adjacent strips from the same blot were probed with either the RB7-6 or the S116-1.1B MAR.

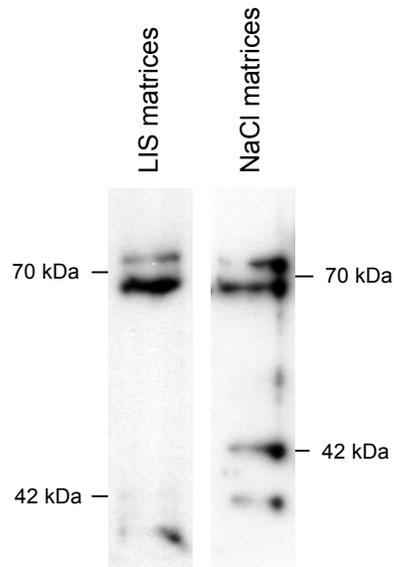


Figure 3-4. Comparison of MAR binding proteins from LIS and NaCl matrices. Matrices from tobacco NT1 culture cell nuclei were prepared using either the LIS protocol or the NaCl protocol. Proteins were separated by electrophoresis on an SDS acrylamide (8%) gel, transferred to PVDF membrane, and probed with  $^{32}\text{P}$  labeled RB7-6 MAR DNA. Identical MAR DNA binding protein profiles were observed.

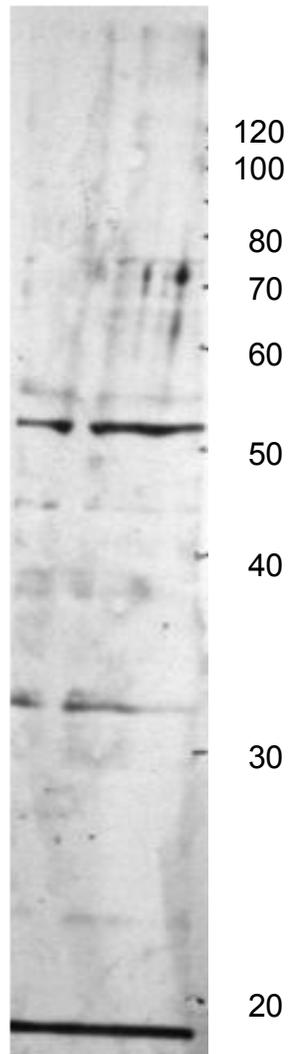


Figure 3-5. Immunoblot of tobacco NT1 cell nuclear matrix protein probed with IFA. Matrix proteins were separated by electrophoresis on an SDS acrylamide (10%) gel and transferred to PVDF. The membrane was probed with IFA, an antibody recognizing a conserved epitope common to all known intermediate filaments. The antibody reacts with a protein at 52 kDa, and reacts weakly with a protein of 33 kDa. This result demonstrates that the MAR binding proteins we have identified are not intermediate filament protein.

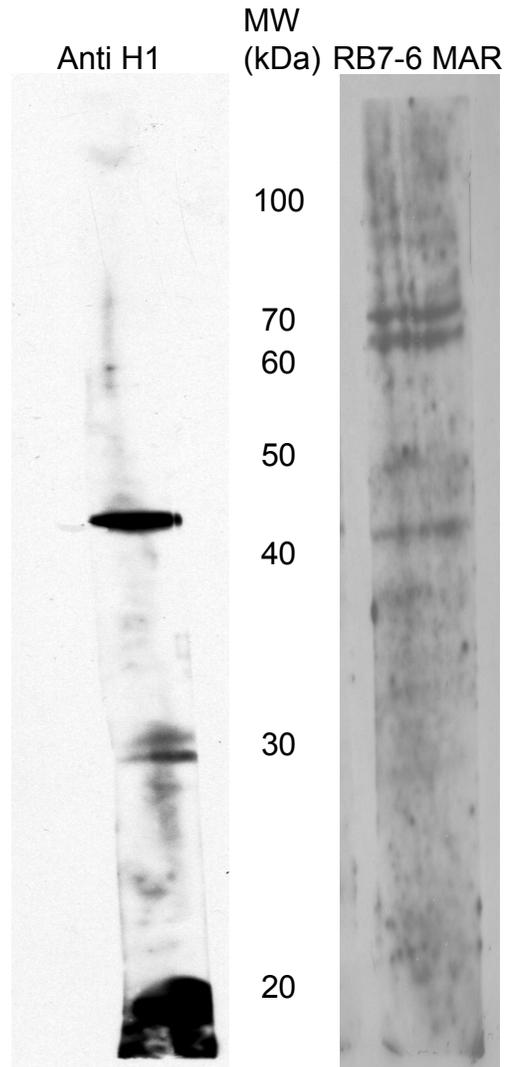


Figure 3-6. Immunoblot of tobacco NT1 matrices probed with polyclonal antibody to wheat histone H1. Tobacco NT1 matrices (LIS) were separated by electrophoresis on an SDS polyacrylamide (10%) gel, transferred to PVDF membrane, and probed with anti wheat H1. A protein migrating at 42 kDa is recognized by the anti-H1 antibody, and a similarly sized protein also binds to the RB7-6 MAR in a southwestern binding assay performed on a strip from the same membrane.

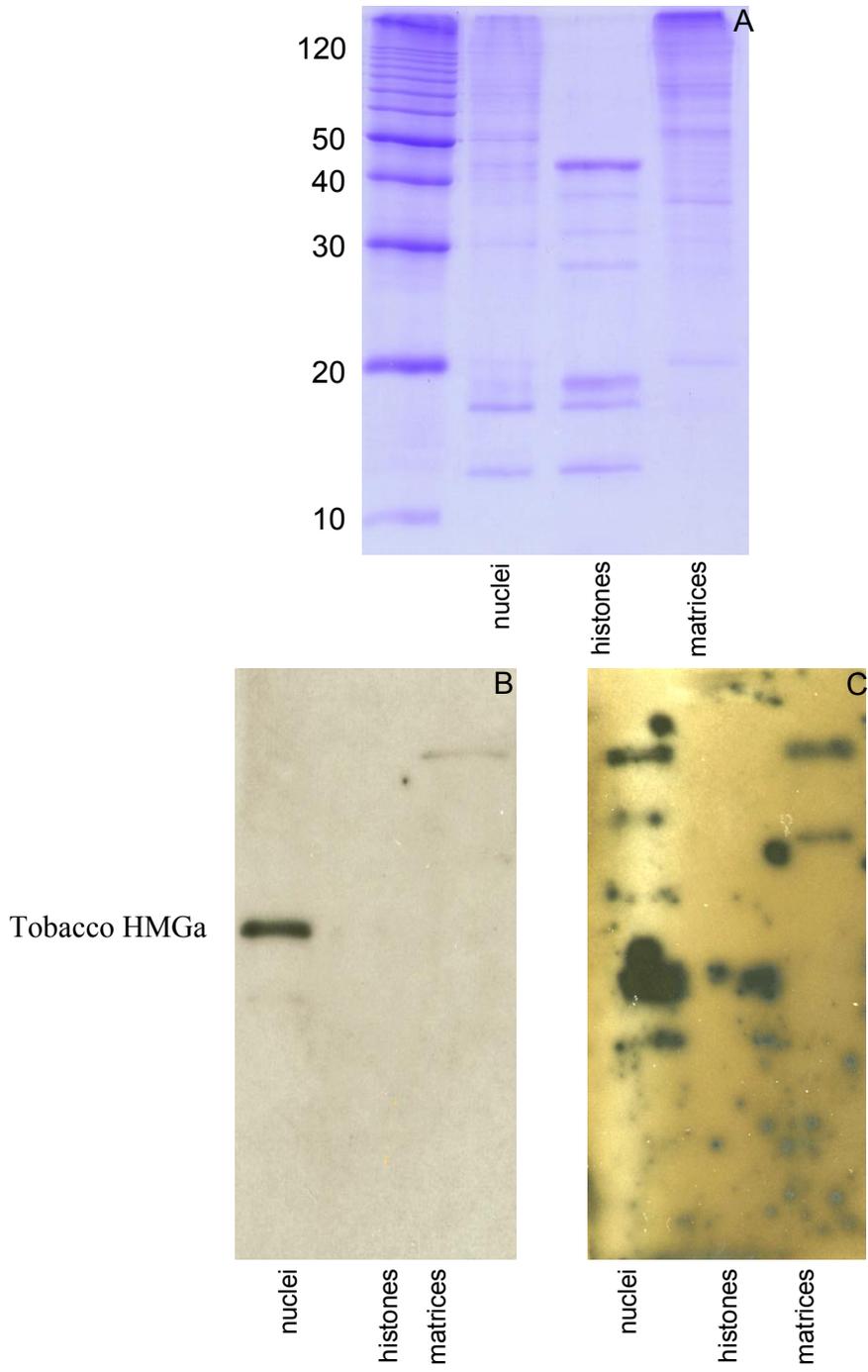


Figure 3-7. Comparison of immunoblot and southwestern blot of tobacco nuclear fractions. (A) NT1 tobacco culture cell nuclear fractions were separated by electrophoresis on an SDS acrylamide (15%) gel. (B) A replicate gel was transferred to PVDF and probed with antisera raised against wheat HMGa protein (Spiker and Everett, 1987). (C) A replicate gel was transferred to PVDF and probed with digoxigenin labeled RB7-6 MAR.