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Dimerization specificity of *Arabidopsis* MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA, and AGAMOUS

(flower development/ABC model/DNA binding/CArG box)

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ABSTRACT **The MADS domain homeotic proteins APETALA1 (AP1), APETALA3 (AP3), PISTILLATA (PI), and AGAMOUS (AG) act in a combinatorial manner to specify the identity of *Arabidopsis* floral organs. The molecular basis for this combinatorial mode of action was investigated. Immunoprecipitation experiments indicate that all four proteins are capable of interacting with each other. However, these proteins exhibit "partner-specificity" for the formation of DNA-binding dimers; only AP1 homodimers, AG homodimers, and AP3/PI heterodimers are capable of binding to CArG-box sequences. Both the AP3/PI heterodimer and the AP1 or AG homodimers are formed when the three corresponding proteins are present together. The use of chimeric proteins formed by domain swapping indicates that the L region (which follows the MADS box) constitutes a key molecular determinant for the selective formation of DNA-binding dimers. The implications of these results for the ABC genetic model of flower development are discussed.**

The study of homeotic mutants in *Arabidopsis thaliana* and *Antirrhinum majus* has led to the establishment of a genetic model (the ABC model) that explains how the fates of floral organ primordia are determined (1, 2). According to the ABC model, the identities of the organs of an *Arabidopsis* flower (four sepals, four petals, six stamens, and two carpels) are specified by the action of at least five organ identity genes, all of which have been cloned: *APETALA1* (*AP1*), *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*) (3–7). *In situ* hybridization and ectopic expression experiments (8–10) have provided strong evidence supporting the ABC model, showing that each whorl of a flower primordium has a unique combination of organ identity activities, which combinatorially specify organ identity. The specification of sepals is dependent on class A gene activities (*AP1* and *AP2*), petals are specified by a combination of class A and class B (*AP3* and *PI*) gene activities, stamens are specified by the combined activities of classes B and C (*AG*), and specification of the carpels is achieved by class C activity.

AP1, AP3, PI, and AG are all MADS domain-containing proteins (Fig. 1), while AP2 bears similarity to a different class of DNA binding proteins (4, 13). The MADS domain is a conserved region of 56 amino acids present in a variety of dimeric transcription factors from different organisms. The MADS regions of SRF and MCM1 have been characterized as DNA binding and dimerization domains (11, 14). Within the family of MADS box proteins, the plant proteins are unique in that they contain another conserved region, the K box, that may form amphipathic alpha helices (ref. 12; Fig. 1). SRF and MCM1 bind to CArG-box sequences [consensus CC(A/T)$_6$GG] (11, 15, 16), and it has been shown that AG binds to such sequences (17–19). The *Antirrhinum* DEFICIENS (*DEF*, the ortholog of *AP3*) and GLOBOSA (*GLO*, the ortholog of *PI*) proteins have been shown to bind together as a heterocomplex, providing a molecular mechanism to explain the requirement for both genes in the B function (20, 21).

While *AP1*, *AP3*, *PI*, and *AG* have been extensively characterized at the genetic level, little is known about the molecular mechanisms by which their protein products act. One possible mechanism by which AP1, AP3, and PI (which specify petals) and AP3, PI, and AG (which specify stamens) could act combinatorially to dictate a developmental program is through heterocomplex formation. Can the four proteins interact with each other? If they interact, are all the different homo- and heterocomplexes capable of binding to DNA? If this is not the case, and the proteins present partner-specificity for the formation of DNA-binding complexes, where in the proteins are the determinants for this partner-specificity localized? Here, we show that AP1, AP3, PI, and AG are all capable of interacting with each other, but that only AP1/AP1, AP3/PI, and AG/AG dimers (and also heterodimers formed by truncated AG and AP1) bind to CArG-box containing sequences. This DNA-binding partner specificity is mediated to a large degree by the L region of these proteins, a 31–35 aa segment located between the MADS and K boxes.

MATERIALS AND METHODS

Plasmid Construction. AP1, AP3, PI, and AG cDNA coding sequences were cloned into the pSPUTK *in vitro* translation vector (Stratagene). Because the initiating ATG codon was not found in the *AG* cDNA clones (7), the wild-type sequence 5′-CATTTT... at the beginning of the *AG* cDNA was changed to 5′-ATGGGG.... The *in vivo* functionality of such an altered AG protein has been shown in ectopic expression experiments (8). The construction of pPI$_{FLAG}$, to make FLAG epitope-tagged PI protein, has been previously described (ref. 6; the sequence KDDDADYKDDDDK was added at the C terminus of the protein). Similar constructs were made for AP1, AP3, and AG. A derivative of *AG*, *AGNML* (to produce a C-terminal truncated protein that comprises the N-terminal amino acids that precede the AG MADS box, and the MADS domain and L region), was synthesized by PCR and cloned into pSPUTK. C-terminal deletion derivatives of AP1, AP3, and PI (AP1$_{M+33}$, AP3$_{ML+31}$, and PI$_{ML+20}$, respectively) were obtained by digesting the plasmids encoding the full-length proteins with internal restriction sites (*Bsr*I, *Alw*NI, and *Bpm*I, respectively) before the *in vitro* transcription reaction. Chimeric constructs were made by synthesizing and fusing cDNA fragments by PCR techniques so that no amino acids (others than those of the domains that were swapped) were changed in the resulting chimeras. Sequences were fused at the end of the MADS box and at the end of the L region (Fig. 1) of the

Abbreviations: AP1, APETALA1; AP2, APETALA2; AP3, APETALA3; PI, PISTILLATA; AG, AGAMOUS; SRF, serum response factor. MCM1, the MEF2 family.

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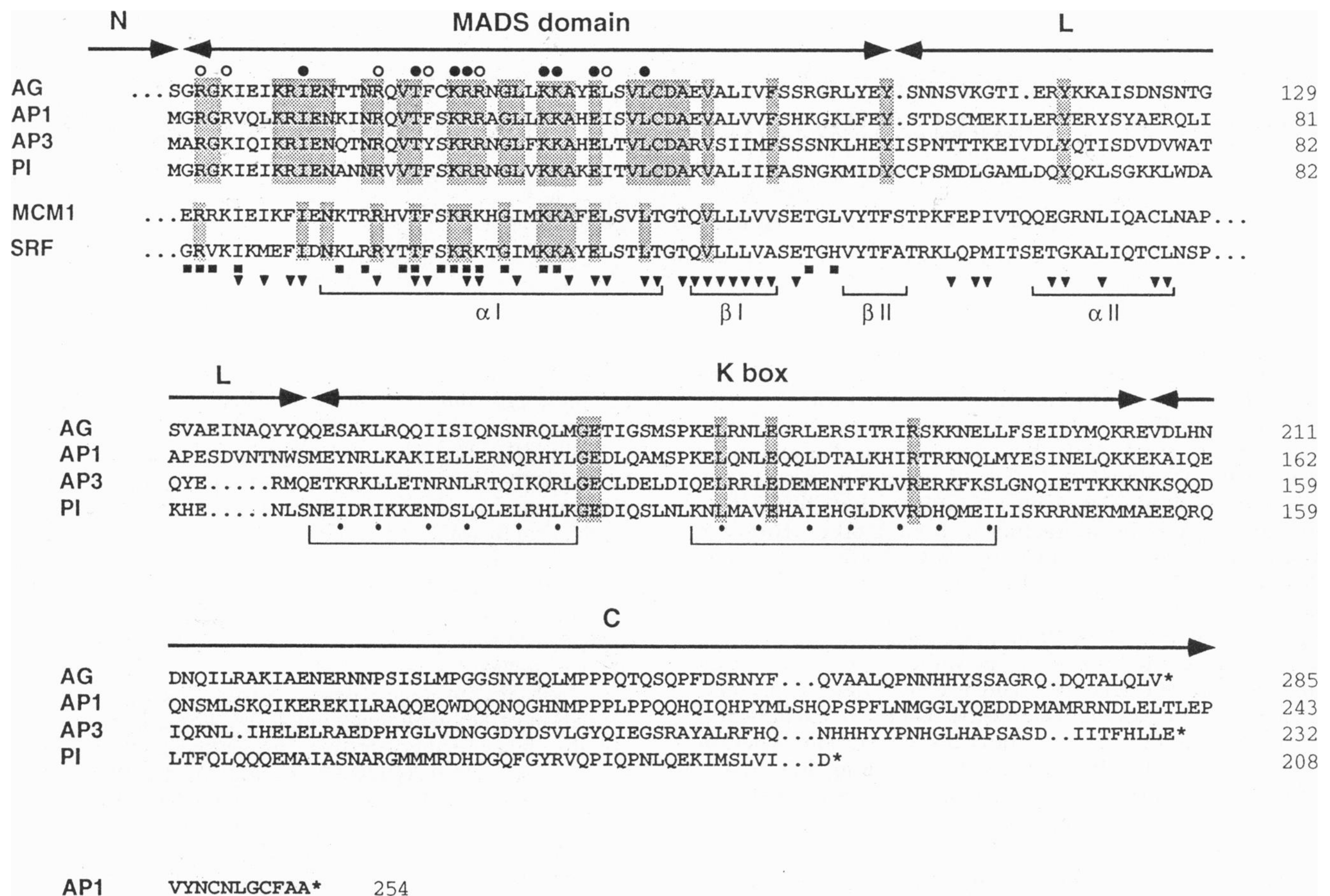

FIG. 1. Alignment of the amino acid sequences of AG, AP1, AP3, and PI. Beneath them are shown the sequences of core serum response factor (SRF) and MCM1. The MADS and K boxes and the N, L, and C regions are indicated; amino acids identical among AG, AP1, AP3, and PI are shaded. Relevant features of the MADS box are indicated; amino acids identical among all the proteins belonging to the MADS box family are noted with filled circles, and those that are highly conserved (S/T, Y/F, L/I, R/K) with open ones. SRF residues that have been found to be involved in DNA binding (■) and dimerization (▲) are indicated (11). αI, βI, βII, and αII indicate secondary structure elements observed in the crystal structure of SRF (11). Two possible helices in the K box are indicated (12), with residues at positions a and d in the coiled coil heptapeptide repeat marked.

corresponding genes, *AG*, *AP3*, and *PI*. The initiation codon for those chimeras that included AG N-terminal sequences was engineered in an *Nco*I context at amino acid residue 34 of wild-type *AG* cDNA (7, 8). The chimeric constructs were cloned into pSPUTK.

DNA-Binding Site Probes. Probes A and B are derived from the promoters of the *Arabidopsis AP3* (22) and *SUPERMAN* (H. Sakai, L. Medrano, and E.M.M., unpublished data) genes, respectively. Probe A: 5′-*ggatcc*TCACTTAGTTTTCATCAACTTCTGAACTTACCTTTCATGGATTAGGCAATACTTTCCATTTTTTAGTAACT*aagctt*-3′; probe B: 5′-*ggatcc*TAAGAAAAATGGGAGAAAGGAACATCCACTTTTCCATTTTTGGTATAAAACTTTTGATATAATATGTCCTTTTGCT*aagctt*-3′; (the CArG-boxes are underlined and the plasmid-derived linker sequences are in lowercase type). Binding probes were labeled with ^{32}P by Klenow fill-in reaction and were purified by PAGE before their use in DNA-binding experiments.

Immunoprecipitation and DNA-Binding Assays. Proteins were synthesized using the TNT reticulocyte lysate system (Promega) or in separate transcription and translation reactions. Labeled [^{35}S]methionine *in vitro* translation reactions demonstrated that the proteins were produced in similar amounts. AP1, AP3, PI, and AG were synthesized as both epitope-tagged unlabeled and native [^{35}S]methionine-labeled proteins for the immunoprecipitation experiments, which were performed as described (6) with slight modifications. In brief, the *in vitro* translation solution was diluted with an equal volume of 2× buffer A (6). The diluted protein solutions (10–30 μl) were mixed and incubated at room temperature for 30–45 min to allow protein association. Ice-cold buffer B (400 μl) (6) and 1.5 μg of anti-FLAG M2 monoclonal antibody (Kodak–IBI) were added and the samples were incubated at 4°C on a rotating wheel for 30 min. Protein A-Sepharose CL4B slurry (100 μl; Sigma, equilibrated in buffer A) was then added and the incubation continued for 1 hr. The sepharose beads were collected by centrifugation and washed 4× with RIPA buffer (50 mM Tris·HCl, pH 8/150 mM NaCl/1% deoxycholate/1% Triton X-100/0.2% SDS/4 mg/ml BSA), once with LiCl/urea buffer (10 mM Tris·HCl pH 8/400 mM LiCl/2 M urea), and once with water. Immunoprecipitated proteins were analyzed by SDS/PAGE on 12.5% gels. *In vitro* translated proteins were tested for DNA-binding activity by electrophoretic mobility-shift assay. The standard binding reaction (final vol of 10 μl) contained 2 μl of the translation mixture, 20 mM Tris·HCl (pH 8), 100 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 12% glycerol, 2.5 μg of BSA, 200 ng of double-stranded poly(dI·dC) (Pharmacia), and 200 ng of sonicated calf thymus DNA (Pharmacia). Reactions were preincubated at room temperature for 25 min and for a further 15 min after the addition of the labeled probe (10,000–20,000 cpm; final concentration of 0.12–0.25 nM). Gels for resolving protein–DNA complexes were 5% polyacrylamide/bisacrylamide (60:1) in 1 × TBE.

RESULTS

Interactions of AP1, AP3, PI, and AG. The ability of these four proteins to interact with each other was investigated by immunoprecipitation. Epitope-tagged AP1, AP3, PI, or AG was mixed with nontagged, radioactively labeled proteins and coprecipitated using the tag antibody. This assay has been used previously to show that AP3 and PI proteins associate in solution (6). Coprecipitation of each labeled protein was observed with all of the tagged proteins (Fig. 2). Labeled AP1 was precipitated by tagged AP1, AP3, PI, and AG at levels well above the background (Fig. 2, lanes 1–5), and similar results were obtained for labeled AP3, PI, and AG (Fig. 2). These results indicate that AP1, AP3, PI, and AG can interact with each other *in vitro*.

DNA-Binding Activity of AP1, AP3, PI, and AG. The ability of these various complexes to bind DNA was investigated. Proteins were synthesized by cotranslation in different combinations and analyzed with probe A by electrophoretic mobility-shift assays, in which the different MADS box protein–DNA complexes are distinguishable by their different mobilities. As expected, the DNA-binding complexes formed by these proteins are dimers (J.L.R. and E.M.M., unpublished results). AP1, AP3/PI, and AG dimers showed DNA-binding activity (Fig. 3*A*, lanes 2, 5, and 8). AP3 and PI homocomplexes did not bind to probe A (Fig. 3*A*, lanes 6 and 7). When either of these two proteins was present in combination with AG, the only band shifts detected were those due to the AG protein alone (Fig. 3*A*, lanes 8–10). Similarly, AP1/AP3 and AP1/PI reactions only showed the bands corresponding to AP1 (Fig. 3*A*, lanes 2–4). The same results were obtained using probe B

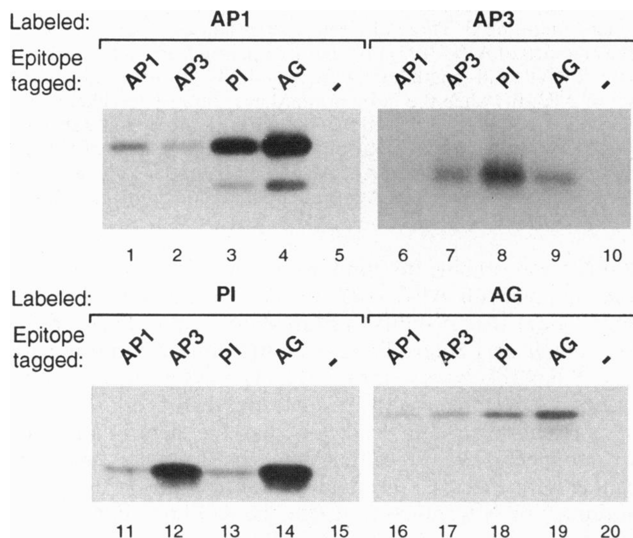

FIG. 2. AP1, AP3, PI, and AG can interact with each other. [35S]-methionine-labeled *in vitro* translated proteins (5 μl) were mixed with *in vitro* translated epitope-tagged AP1 (11 μl), AP3 (15 μl), PI (10 μl), or AG (10 μl), or with unprogrammed lysate (as control for nonspecific precipitation), and subjected to immunoprecipitation with an anti-FLAG monoclonal antibody. The number of methionines in these proteins is AP1, 11; AP3, 4; PI, 13; AG, 6. Lanes 6–10 show a longer exposure of the autoradiogram than the other lanes. The failure to detect coprecipitation of labeled AP3 with tagged AP1 in this experiment (lane 6) resulted from both the weakness of the AP1-AP3 interaction and a presumably low amount of tagged AP1 produced in the translation reaction (this particular coprecipitation was observed in other experiments; not shown). A quantitative comparison of all these interactions is partially precluded, because the amount of each of the tagged proteins may be different and because several reactions (which might not be at equilibrium) coexist in each assay while only one of them is detected. The band with higher mobility in lanes 1–4 corresponds to a truncated AP1 protein that is produced in the translation reaction.

and with additional CArG-box containing probes, or when the proteins were translated separately and mixed afterwards (data not shown). Although AP1, AP3, PI, and AG can interact with each other, these data indicate that the ability to form DNA-binding complexes that recognize the binding sites used is restricted to certain combinations (AP1, AP3/PI, and AG). The interaction between AG and AP1 could not be assayed with the two full-length proteins, because both proteins produce band shifts at similar positions that, in addition, are very different in their intensities. When truncated forms of AG and AP1 were assayed together, a band corresponding to a heterodimer between these two proteins was detected (data not shown). This AP1/AG interaction is probably irrelevant (if existent) *in vivo*, because AG and AP1 are not active in the same cells (3, 7, 23, 24).

Two different protein–DNA complexes were detected when either AP1, AP3, and PI, or AP3, PI, and AG were synthesized by cotranslation and analyzed in electrophoretic mobility-shift assays. The presence of AP1, AP3, and PI together resulted in band shifts corresponding to AP1 homodimers and AP3/PI heterodimers (Fig. 3*B*). Similarly, when cotranslated AP3, PI, and AG were incubated with DNA, simultaneous DNA binding by AP3/PI and AG dimers was detected (Fig. 3*C*). Thus, different DNA-binding MADS box protein complexes can coexist, at least within a certain range of relative protein concentrations. These data suggest that DNA-binding AP1 homodimers and AP3/PI heterodimers are formed in primordial petal cells that express AP1, AP3, and PI, and that DNA-binding AG homodimers and AP3/PI heterodimers form in primordial stamen cells that express AG, AP3, and PI.

Localization of Dimerization-Specificity Determinants. C-terminal deletion analysis of AP1, AP3, PI, and AG indicated that the "core" portion (minimal DNA-binding domain) of AP1 and AG includes the MADS box and part of the L region, whereas AP3 and PI require the first amino acids of the K box, in addition to the MADS domain and L region, to form a DNA-binding complex (J.L.R. and E.M.M., unpublished results). When the "core" proteins were assayed for DNA binding activity in different combinations, it was found that the partner-specificity observed in the full-length proteins is preserved in the "core" derivatives: $AP3_{ML+31}$ (truncated protein that contains the MADS box, L region, and the first 31 amino acids of the K box) and PI_{ML+20} do not form DNA-binding homodimers (data not shown), consistent with the fact that neither AP3 nor PI have such activity (Fig. 3). Reactions that included either AG_{NML} or $AP1_{M+33}$ (which contains 33 amino acids of the 35-aa long L region) and AP3 or PI truncated proteins only showed the bands corresponding to DNA binding by the AG_{NML} and $AP1_{M+33}$ homodimers (data not shown).

To localize the protein region(s) responsible for partner-specificity, chimeric proteins formed by domain swapping between AG, AP3, and PI were used in DNA-binding experiments. BGFN1 hybrid protein contains N-terminal sequences from AG (part of the N-terminal region and the MADS box) fused to the AP3 L, K, and C regions (Fig. 4*B*). BGFN1 bound DNA as a heterodimer with PI, but not with AP1, AP3, or AG (Fig. 4*A*, lanes 2–5). In addition, BGFN1 did not bind to DNA as a homodimer (Fig. 4*A*, lane 1). This result indicates that the determinants for the specificity of the interaction between AP3 and PI reside C terminal to the MADS domain. BGFN2 differs from BGFN1 in that its L region is from AG instead of AP3 (Fig. 4*B*). In contrast with BGFN1, BGFN2 bound DNA as a homodimer and did not interact with PI in a productive (DNA-binding) fashion (Fig. 4*A*, lanes 6–10). The results obtained with BGFN1 and BGFN2 therefore indicate that the L region is a key determinant for formation of partner-specific DNA-binding complexes.

BGFN5 contains the AP3 MADS domain and L region fused to the AG K box and C region (Fig. 4*B*). BGFN5 did not exhibit DNA-binding activity either as a homodimer or in any

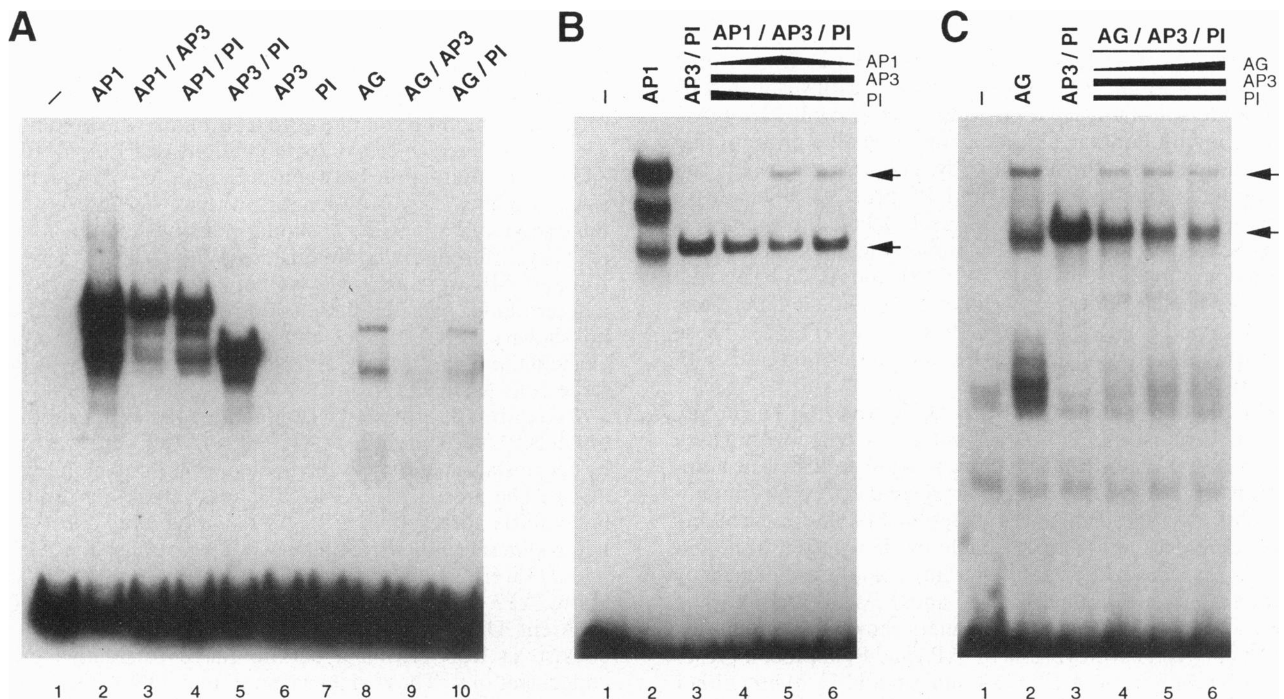

FIG. 3. AP1, AP3, PI, and AG show partner specificity for the formation of DNA-binding complexes. *(A)* Proteins were synthesized by *in vitro* transcription/translation in the indicated combinations and assayed with probe A. The various shifted bands present in the reactions with AP1 and AG correspond to protein–DNA complexes formed by the full-length proteins as well as by truncated proteins also produced in the translation reactions. The intensities of shifted bands corresponding to AP1 and AG complexes are reduced when AP3 or PI are present (lanes 2–4 and 8–10), an effect that could be due both to differences in the amount of protein produced when cotranslated and to titration of some of the AP1 and AG proteins away from DNA-binding homodimers. A control with unprogrammed lysate is included (lane 1). *(B)* AP1 and AP3/PI DNA-binding complexes can coexist. *In vitro* cotranslated proteins were tested for DNA-binding with probe B. Three different cotranslations of AP1, AP3, and PI, varying in the amounts of plasmids used, were assayed (lanes 4–6; the relative amounts of AP1/AP3/PI-producing plasmids in each translation reaction were 0.7/1/0.5, 1.4/1/0.3, and 1/1/0.2). Positions of the shifts originated by AP1 full-length homodimers and AP3/PI heterodimers are indicated by arrows. The strong reduction of AP1 DNA-binding activity (but not of AP3/PI) when the three proteins are cotranslated likely arises from differences in the strength of the interactions among them. Immunoprecipitation experiments indicated that the AP3/PI interaction is stronger than the AP1/PI, and the latter stronger than that of AP1/AP1 (data not shown). *(C)* AG and AP3/PI DNA-binding complexes can coexist. Three different cotranslations of AG, AP3, and PI were assayed with probe B (lanes 4–6; the relative amounts of AG/AP3/PI-producing plasmids in each translation reaction were 1.2/1/0.4, 1.7/1/0.4, and 2.3/1/0.4). Positions of the shifts originated by AG full-length homodimers and AP3/PI heterodimers are indicated by arrows.

combination (Fig. 4*A*, lanes 11–15). The inability of this protein to form a DNA-binding complex with PI is consistent with the defined cores of AP3 and PI, which require the first amino acids of the K box to form a DNA-binding heterodimer (see above), and indicates that this part of the AG protein cannot substitute for the corresponding region in AP3. Thus, in the case of AP3, determinants for partner-specificity, although centered in the L region, extend C terminal to it.

BGFN7 contains N-terminal sequences from AG (part of the N terminal region and the MADS box) fused to the PI L, K, and C regions (Fig. 4*B*). Consistent with the role ascribed to the L region, BGFN7 bound DNA as a heterodimer with AP3 (Fig. 4*A*, lane 18). It was also capable of forming a DNA-binding homodimer (Fig. 4*A*, lane 16) and showed a weak DNA-binding interaction with a truncated AG protein, AG$_{NML}$ (Fig. 4*A*, lanes 21 and 22). The ability of BGFN7 to form a heterodimer with full-length AG could not be determined as the BGFN7 and AG homodimers produce similarly shifted bands. These results indicate that (*i*) the PI L, K, and C regions differ from the corresponding domains of AP3 since they can form a homodimer when fused to an AG MADS box, while those of AP3 cannot (Fig. 4*A*, lane 1) and (*ii*) although the AG MADS box could substitute for the PI one, they are not equivalent since the AG MADS box conferred a new capability to the chimeric protein, that of forming homodimers. Differences between the AG MADS box and the PI MADS box were also indicated by chimeric protein BGFN9, in which a PI MADS box is fused to AG L, K, and C regions (Fig. 4*B*). BGFN9 was unable to bind to DNA, either alone or in combination with AP1, AP3, PI, AG, or AG$_{NML}$ (Fig. 4*A*, lanes 23–27). The inability of BGFN9 to form a DNA-binding homodimer or a heterodimer with AG indicates that the AG MADS box (likely its C-terminal half) plays a role in partner specificity. In summary, the results from the experiments with these chimeric proteins indicate that the determinants for partner-specificity are centered in the L region, but also include sequences C terminal to it (for the AP3/PI heterodimer) or N terminal to it (for the AG homodimer).

DISCUSSION

The results indicate that although AP1, AP3, PI, and AG can interact in solution, they exhibit partner-specificity for the formation of DNA-binding dimers. In addition, they provide evidence for the critical involvement of amino acids C terminal to the MADS box in determining the DNA-binding dimerization selectivity of the MADS domain proteins. This function of the L region is consistent with it being a segment that varies considerably, both in sequence and length (27–42 amino acids), within the plant MADS box family of proteins (25, 26). The involvement of the region C terminal to the MADS box in dimerization selectivity was also suggested from experiments that showed that SRF, MCM1, and ARG80, which share loose sequence similarity in this region, can dimerize with each other *in vitro* (17, 27), whereas SRF cannot dimerize with MEF2 proteins (28) with which it does not share such similarity

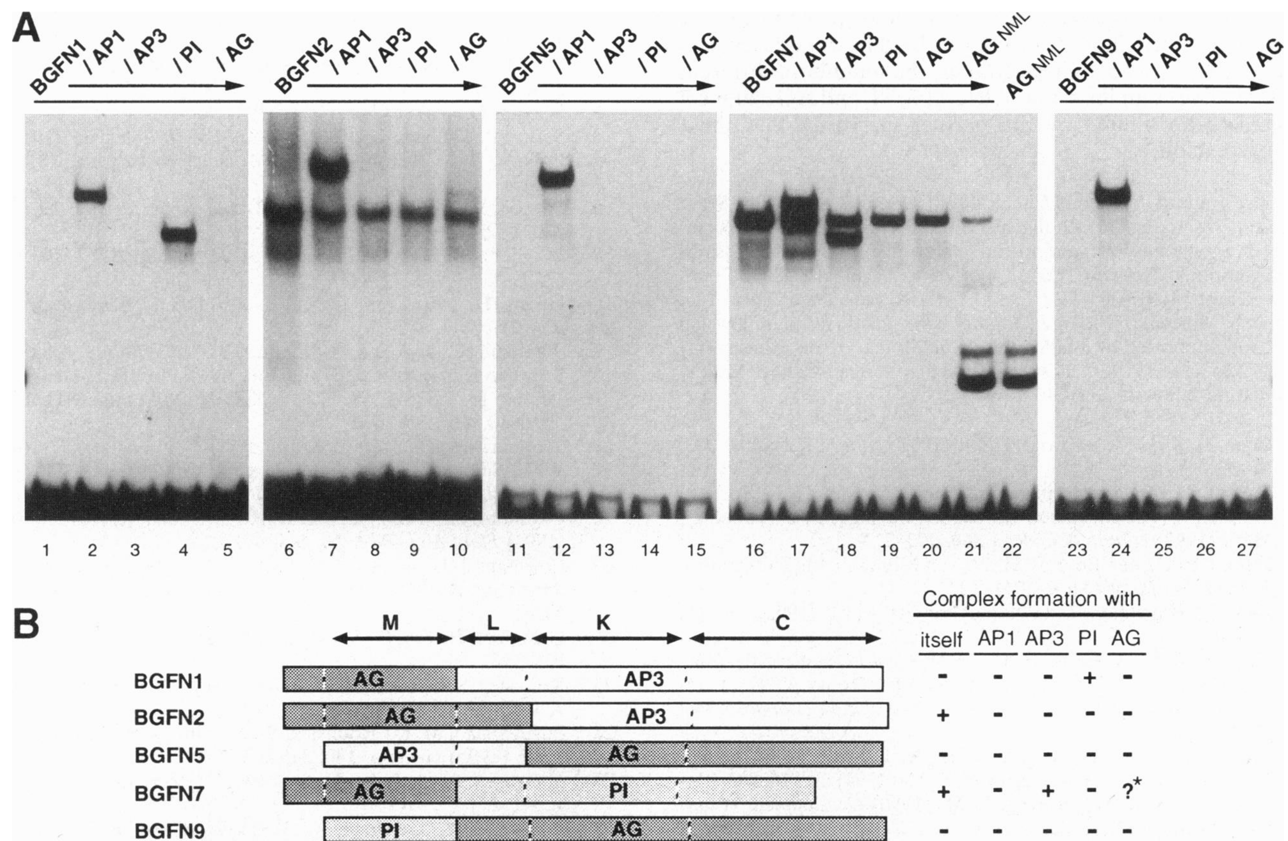

FIG. 4. DNA-binding activity of AP3-AG and PI-AG hybrid proteins. *(A)* Chimeric proteins BGFN1 (lanes 1–5), BGFN2 (lanes 6–10), BGFN5 (lanes 11–15), BGFN7 (lanes 16–22), and BGFN9 (lanes 23–27) were synthesized by *in vitro* translation and assayed for DNA-binding activity by themselves or in combination with AP1, AP3, PI, AG, and AGNML (a truncated AG protein comprising the N terminal region, and the MADS box and L region) using probe A. In lanes 2, 7, 12, 17, and 24, the band corresponding to an AP1 homodimer is seen. The band corresponding to the AG homodimer in lanes 5, 15, and 27 required a longer exposure of the autoradiograms to appear. *(B)* Structure of MADS domain chimeric proteins and summary of the results obtained in the DNA-binding experiments. The question mark and the asterisk assigned to the combination of BGFN7 and AG proteins indicate that such a heterodimer could not have been detected in the experiment, but that a weak interaction between BGFN7 and AGNML was observed.

(14). The recently determined crystal structure of core SRF bound to DNA showed that some of the contacts between the two monomers involve amino acids in this part of the protein (11). The differences in the size of the core proteins and in the regions involved in partner specificity indicate that the dimerization requirements of AP3 and PI are different from those of AG and AP1, perhaps explaining the apparent inability of heterodimers between AP3 or PI and AG or AP1 to bind DNA.

The partner-specificity that AP1, AP3, PI, and AG exhibit has implications for the translation of the ABC genetic model of flower development into a molecular mechanism. At least two different scenarios can be envisioned to account for the combinatorial mode of action of AP1, AP3, PI, and AG. One possibility is that there may be direct interactions between these proteins, therefore forming new complexes with additional regulatory capabilities. Another possible mechanism is that these proteins do not interact directly and it is the various combinations of downstream genes activated or repressed by the AP1, AP3/PI, and AG complexes that determine the identity of an organ. The genetic data that have been obtained during the past years do not allow the validation or rejection of either scenario. The results presented here do not support a functional role for complexes like PI/AG, because AP3 and PI homodimers and AP3/AG, PI/AG, AP3/AP1, and PI/AP1 heterodimers are apparently unable to bind DNA. The results obtained with the chimeric proteins indicate that the inability of certain combinations of AP1, AP3, PI, and AG proteins to form DNA-binding dimers does not arise from different DNA sequence specificities of the MADS boxes. This is exemplified by BGFN1, which showed that a dimer containing an AG MADS box and a PI MADS box recognizes probe A, in contrast with the inability of the AG/PI complex to bind to such a probe. Similarly, a dimer containing an AG MADS box and an AP3 MADS box (BGFN7/AP3) binds to DNA, whereas the AG/AP3 complex does not. Additional possibilities that cannot be excluded on the basis of the *in vitro* experiments are that the protein complexes have a cellular function in the absence of DNA-binding activity, or that these complexes are capable of DNA-binding in the presence of additional proteins. However, *in vivo* data support the idea that at least some of the non-DNA-binding complexes are nonfunctional. It has been shown that *PI* is necessary for the accumulation of AP3 protein (9). An explanation for this result is that AP3 might be stable in the cell only when it is complexed with PI, implying that PI is the only AP3 partner among the four proteins we have studied. The possible dimerization partners for AP1, AP3, PI, and AG are not restricted to these four proteins, however, since the plant MADS box family of proteins is quite large, with over 20 members already identified in *Arabidopsis*, many of which are expressed in flowers (12, 25). Nevertheless, it should be kept in mind that the extensive genetic analyses have so far identified only these four proteins as the products of homeotic genes controlling floral organ identity. In summary, the results obtained with AP1, AP3, PI, and AG do not lend support to the idea that the combinatorial mode of action of the A (*AP1*), B (*AP3* and *PI*), and C (*AG*) gene activities during flower development is achieved through

direct interactions between the corresponding proteins and the concomitant formation of new DNA-binding complexes with novel regulatory capabilities. Rather, the number of different DNA-binding complexes that AP1, AP3, PI, and AG can form appears to be limited by the partner-specificity that these proteins exhibit.

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