Formation of the CRS2-CAF2 Group II Intron Splicing Complex Is Mediated by a 22-Amino Acid Motif in the COOH-terminal Region of CAF2

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CRS2-associated factors 1 and 2 (CAF1 and CAF2) are closely related proteins that function in concert with chloroplast RNA splicing 2 (CRS2) to promote the splicing of specific sets of group II introns in maize chloroplasts. The CRS2-CAF complexes bind tightly to their cognate group II introns in vitro, with the CAF subunit determining the intron specificity of the complex. In this work we show that the CRS2-CAF complexes are stable in the absence of their intron targets and that CRS2 binds a 22 amino acid motif in the COOH-terminal region of CAF2 that is conserved in CAF1. Yeast two-hybrid assays and co-fractionation studies using recombiant proteins show that this motif is both necessary and sufficient to bind CRS2. The 22-amino acid motif is predicted to form an amphipathic helix whose hydrophobic surface is conserved between CAF1 and CAF2. We propose that this surface binds the hydrophobic patch on the surface of CRS2 previously shown to be necessary for the interaction between CRS2 and CAF2.

Group II introns are large, catalytic RNAs defined by a conserved structural organization consisting of six largely helical domains, characteristic interdomain interactions and several limited regions of conserved sequence (1–4). A few group II introns have been shown to self-splice in vitro; however, to do so typically requires non-physiological conditions. In vivo, proteins are required for efficient splicing, presumably to aid the folding of these large, intricately structured RNAs into their catalytically competent conformations (5, 6).

Group II introns are found in bacteria, mitochondria, and chloroplasts, and, sporadically, in archaea (6–9). The majority of bacterial group II introns are predicted to have the canonical group II intron fold and to encode a “maturase” protein that facilitates both splicing and intron mobility (7, 10). In contrast, many organellar group II introns deviate in their primary sequence and predicted structure from canonical self-splicing group II introns, do not encode a maturase protein, and fail to exhibit self-splicing activity. In particular, none of the ~40 group II introns found in the chloroplasts and mitochondria of higher plants have been observed to splice autonomously (11–13). The loss of self-splicing activity and maturase open reading frames from organellar introns was accompanied by the recruitment of host-encoded protein cofactors that are essential for splicing (5, 6, 10, 11).

Chloroplast RNA splicing 2 (CRS2)5 is a nucleus-encoded protein that is required for the splicing of 9 of the 17 maize chloroplast group II introns (13, 14). A yeast two-hybrid screen identified two related proteins that interact with CRS2: CRS2-associated factors 1 and 2 (CAF1 and CAF2) (12). Chloroplast splicing defects in caf1 and caf2 mutants showed that CRS2-dependent introns require the CAFs for splicing and that these introns can be subdivided according to their requirement for either CAF1 or CAF2 (12): the CRS2-CAF1 complex is required for the splicing of four introns, the CRS2-CAF2 complex is required for the splicing of three introns, and two introns require both CAF1 and CAF2.

CRS2 is closely related to peptidyl-tRNA hydrolases (PTH) (15), whereas CAF1 and CAF2 belong to a family of proteins defined by the presence of a recently recognized RNA binding domain named the CRM domain (16). The CRM domain family includes another chloroplast group II intron splicing factor, CRS1 (17), which has been shown to bind its cognate intron, the atpF intron, with high affinity and specificity in vivo (17) and in vitro (18). CRM domains are also found as conserved, stand-alone open reading frames in prokaryotes, as typified by the Escherichia coli protein YbhY. Structural analysis of three eubacterial YbhY orthologs revealed structural similarity to known RNA binding domains and a putative RNA binding surface (16, 19, 20). The CRM domains in CAF1, CAF2, and CRS1 are expected to adopt a structure similar to that of YbhY and to share a similar RNA binding surface (16). CAF1 and CAF2 each harbor two CRM domains and are therefore predicted to interact strongly with RNA, whereas recombinant CRS2 binds poorly and without specificity to its cognate group II intron RNAs in vitro.5 CRS2-CAF complexes are bound tightly to their genetically defined cognate group II introns in vivo, as shown by the ability of anti-CAF antibodies to selectively co-immunoprecipitate CRS2 and their target intron RNAs from chloroplast extract (12). Taken together these data suggest that the CRM domains in the CAF subunit of each CRS2-CAF complex bind intron RNA and recruit CRS2 to specific introns.

The crystal structure of CRS2 and accompanying site-directed mutagenesis experiments identified a hydrophobic surface on CRS2 that is necessary for its interaction with CAF1 and CAF2 (21). In this work we sought to identify the site(s) in the CAFs that bind CRS2. The CAFs can be divided into three regions: an NH2-terminal region upstream of the first CRM domain, a central region consisting of the two CRM domains, and a COOH-terminal region downstream of the second CRM domain that shows little similarity between the two proteins (Fig. 1). We found that CRS2 binds to a 22 amino acid motif in the COOH-terminal region of CAF2 that is similar to a motif in the COOH-terminal region of CRS1. This 22-amino acid motif is predicted to form

5 The abbreviations used are: CRS2, chloroplast RNA splicing 2; CAF1, CRS2-associated factor 1; CAF2, CRS2-associated factor 2; GST, glutathione S-transferase; PTH, peptidyl-tRNA hydrolase; NTA, nitritoltriacetic acid.

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an amphipathic helix whose hydrophobic residues are very highly conserved among CAF1 and CAF2 proteins in both monocot and dicot plants. These results suggest a model in which the hydrophobic face of this amphipathic helix interacts with the hydrophobic surface of CRS2 previously shown to be required for its interaction with CAF1 and CAF2.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Assays—The yeast two-hybrid assay was performed as described previously (12, 21). The CRS2 open reading frame was amplified by PCR using the primers CRS2D and CRS2L (Table 1). The amplification product was cloned into the EcoRI and SalI sites of the pBD-Gal4 Cam plasmid, to generate the "bait" plasmid pBD-CRS2. The CAF2 "prey" plasmid, pAD-CAF2, has the nearly full-length CAF2 sequence fused to the Gal4 activation domain. It and pAD-CAF1(R312Start) and pAD-CAF2(S138Start) were identified in the yeast two-hybrid screen that discovered the CAFs (12). Truncated CAF2 variants were expressed by introducing stop codons into the CAF2 open reading frame using the Stratagene QuickChange mutagenesis kit. The stop codon substitutions were made using the following primer pairs: K354Stop with CAF2K354StopT-CAF2K354StopB, K452Stop with CAF2K452StopT-CAF2K452StopB, K486Stop with CAF2K486StopU-CAF2K486StopL, and K509Stop with CAF2N509StopU-CAF2N509StopL (Table 1). Plasmid pAD-cI, provided with the Stratagene kit, encodes a fragment of the CAF2 protein fused to the Gal4 activation domain. Yeast strains were generated by sequential transformation using the S.c. EasyComp transformation kit (Invitrogen).

Co-purification of CAF2 Domains with GST-CRS2—GST-CRS2 and CAF2 constructs were co-expressed in E. coli using the compatible vectors pEGST and pAC28 (22). CRS2 was subcloned out of pBD-CRS2 and into the EcoRI and SalI sites of pEGST, thereby fusing glutathione S-transferase (GST) to residue 57 of CRS2. The caf2 gene was amplified using the primers CAF2P and CAF2L, which introduced a BamHI site immediately upstream of Gln59 and placed a HindIII site downstream of the native stop codon. The resulting PCR product, CAF2PQ, was cloned into the BamHI and HindIII sites of pET28a (Novagen). Sequences encoding the NH2-terminal region of mature CAF2, residues Gln59 through Arg226, were PCR amplified with primers CAF2P and CAF2NB and cloned into the BamHI and XhoI sites of pET28a. Sequences encoding the middle region of CAF2, amino acids Glu232 through Leu449, were PCR amplified with primers CAF2MU and CAF2I and cloned into the BamHI and XhoI sites of pET28a. Sequences encoding the COOH-terminal region of CAF2, amino acids Arg442 through the native stop codon, were PCR-amplified with primers CAF2CA and CAF2R and cloned into the BamHI and XhoI sites of pET28a. The vector-encoded histidine-T7 antigen tag was fused, in frame, to the NH2 terminus of CAF2, CAF2N, CAF2M, or CAF2C in these constructs. The CAF2 constructs were subcloned out of pET28a and into pAC28 using the XbaI and ClaI sites that flank the multiple cloning sites. E. coli strain BL21

FIGURE 1. Alignment of CAF1 and CAF2 orthologs from maize and Arabidopsis with predicted mitochondrial CAF paralogs in Arabidopsis. Identical residues are shaded in black. Similar residues are shaded in gray. CRM domains are indicated as is the first amino acid encoded by the truncated CAF1 and CAF2 cDNAs identified in the original yeast-two-hybrid screen (CAF1ΔN and CAF2ΔN) (12). CAF2 positions that were mutagenized to stop codons in this study are indicated by a vertical line and CAF2 residue number.
(DE3) Star (Invitrogen) was co-transformed with pEGST-CRS2 and each of the CAF2 constructs pAC28-CAF2, pAC28-CAF2N, pAC28-CAF2M, and pAC28-CAF2C.

Co-expression cultures were grown in LB supplemented with 200 mg liter⁻¹ ampicillin and 100 mg liter⁻¹ kanamycin sulfate to an A₆₀₀nm of 1, induced by the addition of isopropyl β-D-thiogalactopyranoside to a concentration of 0.1 mM, and incubated overnight at 16 °C. Cells were harvested by centrifugation and frozen at −80 °C. Released CRS2 was eluted with 250 mM NaCl, 25 mM Tris-HCl, pH 8.0, 1% glycerol, 5 mM dithiothreitol overnight at 20 °C. Released CRS2 was eluted with Lysis Buffer. The first 2 ml of the elution were applied to a Superdex 200 gel filtration column in Lysis Buffer lacking protease inhibitors and supplemented with 1% glycerol and 5 mM β-mercaptoethanol. The analogous expression system for CRS2 and CAF2 yielded a complex that behaved similarly to this CRS2/CAF1 complex (data not shown). CAF1 interaction was not tested with the GST-CRS2 fusion protein used here for CAF2.

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** CRS2 Binding Motif of CAF2 **

**TABLE 1**

| Substrates | Primers |
|------------|---------|
| CRS2L      | GGGAGGTCTGGATCTTCAAAACCTG |
| CRS2G      | GGGGCCCCATGGATACCCGCC |
| CRS2H      | CCTCAAGAATTCAAAAACCCGTGG |
| CAF2P      | CAGCAGGAGTCCTCCAAAGAAGACCC |
| CAF2Q      | CGATCCAAAGCTGGAATCTGACC |
| CAF2F      | Same as above |
| CAF2NB     | CAACTCGGCTGCTGCTGCC |
| CAF2N1U    | ATGGATCCGAGCGCCTCACCACCATCT |
| CAF2P1     | TATTCGAGTCACACTAGAGGCTGATTCAT |
| CAF2CA     | AATGGGGATCAGAATACAGG |
| CAF2R      | CGAACACTGTCGGAGTACCTACCTA |
| CAF2K545StopT | CCATTCCTCTCAATCTCAAGTGCATTCTCCTAT |
| CAF2K545StopB | GGCCAGATTTCTCTTCTGGAAATCTTGAATGAGTAAGAGG |
| CAF2K545StopU | AGAGAAGTAGATGAACCCCUAGATTTCAATATTTGGAAG |
| CAF2K545StopL | CTTCTACATTTGGAACATTTGCACTTTCTC |
| CAF2N509StopU | GATGACGGCAGGACATAGATCTCACACC |
| CAF2N509StopL | ATCGGGTAGGGATTTCTGGAGTTCGTCGTCACAGT |
| 22merUpper | CTAGATAGTATTTAAACTCTGGAACAGCAGGCTTGAAGAA |
| 22merLower | TCAGACGGGCTTCGGCTGCAAGTTCACAGCAGGGAG |

**RESULTS**

The CRS2-CAF Complexes Are Stable in the Absence of Their Intron Substrates—Previously we showed that CRS2 co-immunoprecipitates with both CAF1 and CAF2 from chloroplast extract and that the immu-
noliprecipitates include cognate group II intron RNAs (12). To determine whether the interaction between the CAFs and CRS2 is stable in the absence of intron RNA, CRS2 was assayed for its ability to form complexes with CAF1 and CAF2 when the proteins were co-expressed in *E. coli*.

CAF2 was co-expressed with either a GST-CRS2 fusion protein or GST alone; cell extracts were applied to glutathione-Sepharose and eluted with reduced glutathione. CAF2 was retained by glutathione-Sepharose when co-expressed with GST-CRS2 but not when co-expressed with GST alone (Fig. 2), indicating that the CRS2-CAF2 interaction is stable in the absence of intron RNA.

To address the analogous question for CAF1, a CRS2 fusion protein with the chitin-binding domain at its NH2-terminus was co-expressed in *E. coli* with CAF1 fused to a hexahistidine tag. The bacterial lysate was subjected to nickel-affinity chromatography followed by chitin-affinity chromatography. After release of CRS2 from the chitin-binding domain by intein-mediated cleavage, the eluted material was applied to a gel filtration column. CRS2 and CAF1 co-eluted from the column at a position corresponding to globular proteins of ~150 kDa (Fig. 3), whereas CRS2 expressed alone eluted with globular proteins of ~22 kDa (data not shown), which corresponds to its monomeric molecular mass. These results show that the CRS2-CAF1 complex is stable in the absence of intron RNA. The sum of the molecular weights of mature CAF1 and CRS2 is ~90 kDa; elution from the gel-filtration column with globular proteins of ~150 kDa indicates that the CRS2-CAF1 complex is either non-globular and/or that it includes more than one molecule of CAF1 or CRS2.

**A 22-Amino Acid Segment of the CAF2 COOH-terminal Region Is Required for CRS2 Interaction in Yeast**—The original yeast two-hybrid screen that revealed the interaction of CRS2 with CAF1 and CAF2 recovered CAF1 and CAF2 cDNAs that were truncated at their 5’ ends (Figs. 1 and 4A) (12); the CAF1 truncation is particularly extensive, removing nearly half of the protein. Assuming that CAF1 and CAF2 bind CRS2 in a similar manner, the recovery of this truncated CAF1 cDNA suggested that the CRS2 binding site is located in the carboxy-terminal region of both CAF1 and CAF2.

A yeast two-hybrid assay involving a series of CAF2 variants was used to define the region of CAF2 required for its interaction with CRS2. The CAF2 open reading frame was truncated by introducing stop codons immediately downstream of the first CRM domain (K354Stop), the second CRM domain (K452Stop) and in the carboxy-terminal region (N509Stop) (Figs. 1 and 4A). CAF2(N509Stop) interacted with CRS2 but CAF2(K354Stop) and CAF2(K452Stop) did not (Fig. 4B). As such, the 57 amino acids between Lys452 and Asn508 are required for CAF2 to bind CRS2.

The COOH-terminal regions of CAF1 and CAF2 show similarity only within two short segments: their COOH-terminal 16 residues and the segment encompassing CAF1 residues 556–589 and CAF2 residues 487–508 (Fig. 1). The latter motif maps within the 57-amino acid segment shown above to be required for CRS2 interaction, suggesting that it might be involved in binding CRS2. To test this possibility, CAF2 truncated at residue 486 was tested for interaction with CRS2 in the yeast two-hybrid assay. Whereas CAF2(N509Stop) did interact with CRS2 CAF2(K486Stop) did not (Fig. 4B). Therefore, CAF2 residues 487–508 are required for the interaction of CAF2 with CRS2.

**CAF2 Residues 487 through 508 Are Sufficient to Bind CRS2**—To define regions within CAF2 that are sufficient to bind CRS2, the NH2-
CRS2 Binding Motif of CAF2

FIGURE 5. The COOH-terminal region of CAF2 is sufficient to bind CRS2. A. CAF2 fragments tested for interaction with GST-CRS2. B. GST-CRS2 was co-expressed with CAF2N, CAF2M, or CAF2C and purified by affinity chromatography to glutathione-Sepharose. GST-CRS2 was detected with an anti-CRS2 antibody (15), and the CAF2 proteins were detected with the anti-T7 tag antibody (Promega). Equal fractions of each sample were analyzed.

FIGURE 6. CRS2 binds CAF2 amino acid residues 487–508. A. Ni2+ affinity chromatography of extracts of E. coli expressing CRS2 together with either NusA-6xHis or NusA-22mer-6xHis, in which CRS2 residues 487–508 are fused to NusA. Proteins were separated by SDS-PAGE and transferred to nitrocellulose. NusA was detected by staining the filter with Ponceau S, and CRS2 was detected by probing with α-CRS2 antibody. Lane 1, molecular mass markers; lane 2, crude lysate; lane 3, cleared lysate; lane 4, Ni2+-NTA flow-through; lanes 5–14, fractions eluted from Ni2+-NTA with an imidazole gradient. B, gel-filtration chromatography of nickel-affinity extract from E. coli co-expressing CRS2 with either NusA-6xHis or NusA-22mer-6xHis. Proteins were separated by SDS-PAGE and visualized by staining with Coomassie Blue. M, molecular mass standards; lane 1, cleared lysate; lanes 2–4, elutions from Ni2+-NTA resin; lanes 5–13, gel-filtration fractions 8–17. Fractions corresponding to the elution of marker proteins alcohol dehydrogenase and bovine serum albumin are indicated.

terminal region of CAF2, its central CRM domain-containing region, and its COOH-terminal region were assayed for their ability to co-purify with a GST-CAF2 fusion protein after co-expression in E. coli and glutathione affinity chromatography (Fig. 5). The COOH-terminal region of CAF2 co-purified with GST-CAF2, whereas the central and NH2-terminal regions did not (Fig. 5B). Thus, the COOH-terminal region of CAF2 is both necessary and sufficient for binding CRS2.

As discussed above, amino acids 487–508 in the COOH-terminal region of CAF2 are necessary to bind CRS2 in yeast, and they are conserved in CAF1. To determine whether these residues are sufficient to bind CRS2, a fusion protein was expressed consisting of NusA fused at its COOH terminus to CAF2 residues 487–508, followed by a vector-encoded COOH-terminal hexahistidine tag (NusA-22mer-6xHis). Untagged CRS2 was co-expressed in E. coli with either NusA-6xHis or NusA-22mer-6xHis. The cell lysates were subjected to Ni2+ affinity chromatography. Upon elution with an imidazole gradient, the highly expressed NusA proteins eluted in a broad peak (Fig. 6A). When co-expressed with NusA-6xHis, little CRS2 bound to the nickel column, and the elution of the small amount of retained CRS2 was diffuse and did not correlate with the NusA peak (Fig. 6A). In contrast, when co-expressed with NusA-22mer-6xHis, more CRS2 bound to the nickel resin, and the elution profile of CRS2 was similar to that of NusA-22mer-6xHis (Fig. 6A). These results suggested that CAF2 amino acids 487–508 are sufficient to bind CRS2.

To further test this possibility, lysates from the same cells were fractionated by gel-filtration chromatography after elution from the Ni2+ resin. When CRS2 was co-expressed with NusA-6xHis, insufficient CRS2 was retained on the nickel resin to observe after gel filtration (Fig. 6B). In contrast, when co-expressed with NusA-22mer-6xHis, the elution profiles of CRS2 and NusA-22mer-6xHis from the gel-filtration column were nearly identical and matched the elution profile for globular proteins of ~80 kDa (Fig. 6B). CRS2 expressed alone elutes in fractions corresponding to its monomeric molecular mass of 22 kDa (data not shown). Taken together, these results provide strong evidence that the 22-amino acid motif from CAF2 is sufficient to bind CRS2.

The 22-Amino Acid CRS2-binding Motif Is Predicted to Form an Amphipathic Helix with a Surface That Is Conserved in CAF1 and CAF2—Fig. 7A shows an alignment between the 22-amino acid CRS2-binding motif of CAF2 and the corresponding regions of CAF1 and of the predicted CAF1 and CAF2 orthologs in Arabidopsis and rice. Residues corresponding to CAF2 Leu199, Ala203, Ser207, Ala209, Leu201, and Leu203 are almost absolutely conserved among the maize, rice, and Arabidopsis CAF1 and CAF2 proteins, and those corresponding to CAF2 Met287, Phe288, Trp295, Val295, and Leu202 are conserved hydrophobic among all six proteins (Fig. 7A). When the CAF2 22-amino acid motif is modeled as an α helix, most of these conserved residues cluster on one face, where they contribute to a broad hydrophobic surface (Fig. 7B). It seems plausible that adjacent regions of this predicted hydrophobic surface mediate two interactions: (i) the absolutely conserved residues...
Leu^{490}, Ala^{494}, Ser^{497}, and Leu^{501} cluster together and are excellent candidates for mediating the interaction with the hydrophobic CAF-binding surface identified previously on CRS2 (21); (ii) the conserved hydrophobic residues 488, 491, 495, and 502 cluster together and could interact with the hydrophobic core of the CAF protein itself. The remaining residues are mostly non-conserved and hydrophilic and would cluster on the opposite face of such a helix. It seems likely that these residues form a solvent-exposed surface of an amphipathic $\alpha$ helix in the CAF-CRS2 complexes.

**DISCUSSION**

CRS2-CAF complexes are required for the splicing of 9 of the 17 maize chloroplast group II introns in vivo. The presence of predicted CRS2 and CAF orthologs in rice and Arabidopsis suggests that orthologous splicing complexes are a general feature of the chloroplasts of higher plants. The evolution of CRS2 from a bacterial PTH required that it gain the ability to bind to novel RNA substrates and to its CAF protein partners. Through a combination of structure determination and mutagenesis studies, the surface of CRS2 that binds the CAFs was identified previously (21). This surface is strikingly more hydrophobic and less polar than the corresponding surface of E. coli PTH. As the folding differences between CRS2 and PTH are minimal, it appears that CRS2 evolved its CAF recognition surface via the substitution of hydrophobic residues for surface exposed hydrophilic residues of its progenitor PTH.

Because the same hydrophobic surface on CRS2 binds both CAF1 and CAF2 (21), it is reasonable to expect that CAF1 and CAF2 will share similar hydrophobic surfaces that dock with CRS2. In addition, since the CRS2 hydrophobic surface is conserved between maize and Arabidopsis (21), it follows that the CRS2 binding surface of the CAFs would likewise be conserved among plant species. In this work we have identified a predicted surface that is common to CAF1 and CAF2 and conserved between monocots and dicots, which could dock into the CAF-binding surface of CRS2.

The CAFs belong to a protein family defined by the presence of CRM domains (12). CRM domains have been proposed to bind RNA (12, 16, 17) and, indeed, an isolated CRM domain binds RNA in vitro. Another member of this family is CRS1, which is required for the splicing of the group II intron in the chloroplast atpF gene (13, 14). A biochemical study has shown that CRS1 promotes the folding of its target intron through specific, high affinity interactions (18). As the CRS2-CAF complexes bind their target introns with high affinity in vitro (12), it is likely that the CRS2-CAF complexes likewise promote the folding of their target introns through specific, high affinity interactions mediated by the CRM domains in the CAF subunits.

The presence in CRS2 of an extensive basic surface and a carboxyl terminus that is rich in basic and aromatic residues suggests that the function of CRS2 in splicing involves binding to its intron targets (21). However, isolated CRS2 does not bind with high affinity to intron RNA in vitro, leading us to propose that the CAFs recruit CRS2 to specific introns (12). In this work we have provided evidence that the CAFs bind CRS2 via a highly conserved amphipathic $\alpha$ helix that is embedded in their generally non-conserved carboxyl-terminal segments. This lack of conservation suggests that the carboxyl-terminal regions of the CAFs serve merely to tether CRS2 to the CAF-intron complexes. Such tethering presumably increases the effective concentration of CRS2 and facilitates its binding to intron RNA. Whether CRS2 functions solely to drive the folding of the intron into its catalytically active conformation or plays some additional role remains to be determined.

In addition to predicted CAF1 and CAF2 orthologs, the Arabidopsis and rice genomes encode two predicted CRM domain proteins that are similar to CAF1 and CAF2 but that are predicted to be targeted to mitochondria (Fig. 1). These proteins terminate immediately after their second CRM domain and therefore lack a region corresponding to the CRS2 binding site described here. In addition, Arabidopsis possesses an uncharacterized gene, At5g19830, that encodes a CRS2-like protein that is predicted to be targeted to mitochondria (21). This protein includes the basic aromatic carboxyl-terminal tail found in CRS2 but not in bacterial PTH (15); however, the residues implicated in CAF binding are not conserved between CRS2 and At5g19830 (21). Therefore, the predicted mitochondrial CAF-like and CRS2-like proteins both lack regions corresponding to those responsible for CRS2-CAF complex formation in the chloroplast. It will be interesting to learn whether these CAF-like and CRS2-like proteins are required for the splicing of mitochondrial group II introns. If the mitochondrial CAF and CRS2 paralogs facilitate splicing without interacting directly, then the CRS2 paralogs can either bind to intron RNA independently or there is an alternative mechanism for their recruitment to intron RNA.

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**FIGURE 7. Conservation of predicted CRS2 binding surfaces among CAF1 and CAF2 in maize, rice, and Arabidopsis.**

A, alignment of maize CAF2 residues 487–508 with the corresponding residues of maize CAF1 and rice and Arabidopsis CAF1 and CAF2. Identical residues are shaded black. Similar residues are shaded gray. Highly conserved residues are indicated with the maize CAF2 residue number.

B, helical wheel analysis of CAF2 residues 486–503. Hydrophobic residues are shaded in gray. Residues whose identities are very highly conserved between CAF1 and CAF2 in maize, rice, and Arabidopsis are circled in bold. Residues that are conserved hydrophobic among CAF1 and CAF2 in all three species are boxed. Residue numbers correspond to maize CAF2.
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