A Pentatricopeptide Repeat Protein Is a Site Recognition Factor in Chloroplast RNA Editing*•

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In higher plants, RNA editing is a post-transcriptional process that converts C to U in organelle mRNAs. We have previously shown that an Arabidopsis thaliana crr4 mutant is defective with respect to RNA editing for creating the translational initial codon of the plastid ndhD gene (the ndhD-1 site). CRR4 contains 11 pentatricopeptide repeat motifs but does not contain any domains that are likely to be involved in the editing activity. The green fluorescent protein fused to the putative transit peptide of CRR4 targeted the plastid. The recombinant CRR4 expressed in Escherichia coli specifically bound to the 25 nucleotides of the upstream and the 10 nucleotides of the downstream sequences surrounding the editing site of ndhD-1. The target C nucleotide of this editing is not essential for the binding of CRR4. Taken together with the genetic evidence, we conclude that the pentatricopeptide repeat protein CRR4 is a sequence-specific RNA-binding protein that acts as a site recognition factor in plastid RNA editing.

RNA editing is a post-transcriptional process that alters the genetic information held at specific sites on an RNA molecule. It has been detected in a variety of organisms, including viruses, fungi, plants, and mammals (1, 2). In plants, RNA editing is the process of altering a specific C nucleotide to U and, less frequently, from U to C in mitochondria and plastids (3–5). The chloroplast genome of higher plants contains about 30 editing events (22). Positional cloning revealed that the Arabidopsis crr4 mutant is defective in the post-transcriptional processes of organelle mRNAs. Maize (Zea mays) CRP1 is required for the translation of petA and psaC and for the processing of petD (25, 26), whereas Arabidopsis CRR2 is required for intergenic RNA processing between rps7 and ndhB (27). Arabidopsis HCF152 is essential for splicing of petB and intergenic RNA cleavage between psbH and petB (28, 29). Arabidopsis PGR3 is involved in the stabilization of petL RNA operons and might be also involved in the translation of petL and one of the ndh genes (30). Members of the PPR protein family are also involved in the

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1 The abbreviations used are: PPR, pentatricopeptide repeat; GFP, green fluorescent protein; GST, glutathione S-transferase.

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suppression of cytoplasmic male sterility (CMS) via RNA processing or editing of CMS-associated transcripts in petunia (*Petunia hybrida*) (31), radish (*Raphanus sativus*) (32), and rice (33, 34). Our genetic results suggest that a PPR protein is a trans-acting factor that is essential for the recognition of the RNA editing site. To conclude this idea, it is essential to show that CRR4 interacts directly with the sequence surrounding the editing site of ndhD-1.

In the present study, we show that the recombinant CRR4 expressed in *Escherichia coli* specifically binds to the sequence surrounding the editing site of ndhD-1. Our data support a model of plastid RNA editing in which PPR protein acts as a trans-acting factor.

**EXPERIMENTAL PROCEDURES**

**Plant Transformation**—The nucleotide sequence encoding the transit peptide of CRR4 was amplified from the genomic DNA of *A. thaliana* (ecotype Columbia gl1) by PCR using primers 5′-TCTAGAATGGAGTTGATTTCACTC-3′ and 5′-CTATAGCGCGAAATCGGCGAGATA-3′. The amplified DNA fragment was ligated to the sGFP (S65T) gene. The resultant chimeric gene was finally cloned into the pBIN19 vector and introduced into Columbia gl1 via *Agrobacterium tumefaciens* MP90.

**Microscopy**—The fluorescence of green fluorescent protein (GFP) was monitored using a BX-8000 fluorescence microscope (Keyence, Osaka, Japan).

**Plasmid Construction for Expressing CRR4 in *E. coli***—The CRR4 sequence corresponding to the putative mature protein was amplified by PCR using the primers 5′-GAATTCGCTTT-GGCTCCTTTCTCGAC-3′ and 5′-CGCCGCAGCAATGTA-CTGGAAACAACAATG-3′. The PCR product was ligated into the pGEX-6P-1 vector (GE Healthcare). The plasmid that encodes CRR4 fused with glutathione S-transferase (GST) at the N terminus was introduced into *E. coli* BL21(DE3).

**Expression and Purification of the Recombinant CRR4 Protein**—Cells cultured at 37 °C in 2.5 liters of LB medium were cooled to 22 °C. Thirty minutes after the temperature shift, expression of the recombinant protein was induced by the addition of 0.4 mm isopropyl-β-d-thiogalactopyranoside at 22 °C for 5–6 h. The cells were harvested and resuspended in buffer comprising 50 mm Tris-HCl (pH 7.5), 0.3 mm NaCl, 7 mm β-mercaptoethanol, 5% glycerol, and 1 mm phenylmethylsulfonyl fluoride. The following steps were performed at 4 °C. The cells were disrupted by sonication and centrifuged at 15,000 × g for 30 min. The supernatant was loaded onto a GSTrap FF column (GE Healthcare) that had been equilibrated with buffer comprising 10 mm sodium-potassium phosphate buffer (pH 7.4), 140 mm NaCl, 2.7 mm KCl, and 4.0 mm β-mercaptoethanol. The recombinant CRR4 protein was eluted with the same buffer containing 10 mm reduced glutathione. The eluted proteins were pooled, and the buffer was exchanged (to 20 mm Hepes-KOH (pH 7.9), 60 mm KCl, 12.5 mm MgCl₂, 0.1 mm EDTA, 17% glycerol, and 2 mm dithiothreitol) by gel filtration using a HiTrap desalting column (GE Healthcare) and finally concentrated using a centrifugal concentrator. The protein concentration was determined by the method of Bradford. Bovine serum albumin was used as the standard.

**Preparation of RNA Probes**—The *Arabidopsis* chloroplast DNA sequences were amplified by PCR with the primers 5′-CAAAAGGATCTCTATAGGAGATAAAG-3′ and 5′-TATGAACCGCAGATATTGGG-3′ for RB1 and 5′-GTTIATTTCTCAGC-ATAGC-3′ and 5′-TCGTATAGAAAGTTCCATC-3′ for RB2. The RB3 sequence was synthesized as a synthetic oligonucleotide. The DNA fragments were then used as templates for *in vitro* transcription using T7 RNA polymerase (Roche Applied Science). For this purpose, the T7 promoter sequence (ATGAATACGACTCACTATAGGG) was attached to the 5′-end of the forward primer. The 5′-ends of the synthesized RNAs were chemically labeled with fluorescein maleimide as described in the instruction manual for the 5′ EndTag™ Nucleic Acid Labeling System (Vector Laboratories, Burlingame, CA). The 5′-labeled RNA probes were then purified through 6–8% polyacrylamide gel containing 8 M urea. The competitor RNAs were also prepared by *in vitro* transcription using T7 RNA polymerase but not labeled with fluorescein maleimide. The purified competitor RNAs and yeast tRNAs were loaded onto a 6–8% polyacrylamide gel containing 8 M urea, electrophoresed, and stained with ethidium bromide. The intactness and concentration of competitor RNAs were then verified by comparing them with a range of yeast tRNA concentrations.

**Electrophoretic Mobility Shift Assay**—A binding reaction was carried out by mixing the various amounts of recombinant CRR4 with the 5′-labeled RNA probe (0.5 nm) in a total volume of 20 μl of solution containing 5 mm Hepes-KOH (pH 7.9), 7 mm MgCl₂, 2.5 mm dithiothreitol, 25 mm KCl, 4.3% glycerol, 0.25 mm EDTA, and 30 fmol of yeast tRNA. Nonlabeled competitor RNAs were preincubated with the protein for 5 min, and then the labeled RNA was added. The reaction mixture was incubated for 15 min at 25 °C. The samples were then loaded onto a 6–8% non-denaturing polyacrylamide gel (acrylamide:N,N'-methylene bisacrylamide at 29:1) and electrophoresed in TBE buffer (89 mm Tris, 89 mm boric acid, and 2 mm EDTA at 4 °C). After electrophoresis, the RNA was transferred onto a nylon membrane. The signals from the labeled RNA were detected using a Gene Image CDP-Star detection kit (GE Healthcare). Signals from the labeled RNA were quantified using a LAS1000 chemiluminescence analyzer (Fuji Film, Tokyo). The *K*_d value was determined from the concentration of protein at which 50% of the RNA probe bound.

**RESULTS**

**Localization of GFP Fused with the Predicted Transit Peptide Sequence of CRR4**—Physiological characterization indicated that the *crr4* defect is specific to the chloroplast NAD(P)H dehydrogenase complex (21). If CRR4 were directly involved in the RNA editing of ndhD-1 in plastids, CRR4 would be imported to the plastids. The ChloroP 1.1 program predicts that the N-terminal 45 amino acids of CRR4 are a transit peptide to the plastids (Fig. 1A). To confirm this prediction experimentally, we constructed transgenic plants that overexpress the N-terminal 59 amino acid residues of CRR4 fused to GFP under the control of cauliflower mosaic virus 35S promoter. The GFP signal was detected exclusively in the plastids of transgenic
plastids surrounding the editing site of ndhD-1. This fact is consistent with our model in which CRR4 binds to the cis-acting element for the RNA editing of ndhD-1 (Fig. 2B). To investigate this possibility, we attempted to express the recombinant CRR4 in E. coli. The 551-amino acid sequence of CRR4 was compared. We used nonlabeled RB1 and RB2 probes as competitor RNAs. The RB2 probe includes the 201 nucleotides of the coding region of ndhD, which is unlikely to contain the cis-acting element for the RNA editing of ndhD-1 (Fig. 2B).

To demonstrate the sequence specificity of CRR4, it is essential that the competitor RNAs used in this assay were accurately quantified. We therefore verified that two competitor RNAs (cold RB1 and cold RB2) were intact and that their concentrations were accurately quantified by comparing them with a range of yeast tRNA concentrations (Fig. 2D). The addition of 100-fold the amount of nonlabeled RB2 probe had no effect on CRR4-RB1 binding (Fig. 2E, left panel). In contrast, the addition of 10-fold the amount of nonlabeled RB1 probe resulted in the disappearance of the retarded band (Fig. 2E, right panel). These results indicate that CRR4 specifically binds to the 151 nucleotides surrounding the editing site of ndhD-1.

CRR4 Is a Trans-acting Factor Required for RNA Editing of ndhD-1—Both in vivo and in vitro analyses of cis-acting elements indicated that fewer than 20 nucleotides of the upstream sequence and, in some cases, fewer than 10 nucleotides of the downstream sequence surrounding the editing site are enough for site recognition for RNA editing (13, 15, 17). The putative trans-acting factor specifically binds to this cis-acting element. If CRR4 really is a trans-acting factor, the short sequence surrounding the editing site of ndhD-1 might be enough to recruit CRR4. To study this possibility, RNA binding activity was determined using an RB3 probe that includes the 25 upstream nucleotides and the 10 nucleotides in the downstream sequence surrounding the ndhD-1 editing site (Fig. 3A). As shown in Fig. 3B (lanes 1–6), the retarded band was detected with increasing amounts of the recombinant CRR4. The presence of about 3–10-fold the amount of CRR4 to the RB3 probe was required for retardation, the same as when using the RB1 probe (Fig. 2C). It was also verified that this binding does not depend on GST (Fig. 3B, lanes 7–9). The addition of 3–10-fold the amount of nonlabeled RB3 probe resulted in the disappearance of the retarded band (Fig. 3C, left panel). However, the addition of excess amounts of nonlabeled RB2 probe had no

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FIGURE 1. Cellular localization of GFP fused with the transit peptide of CRR4. A, predicted domain structure of CRR4. The transit peptide to the plastid is shown by a white box. The PPR motifs are shown as shaded boxes. The E motif is underlined. The C-terminal conserved 15-amino acid motif is shown by an asterisk. B, fluorescence analysis under a microscope. Transgenic Columbia gl1 overexpressing GFP fused with the transit peptide of CRR4 (tp-GFP), transgenic Columbia overexpressing GFP (GFP), GFP fused with the transit peptide of chloroplast L12 ribosome protein (cp-GFP), and GFP fused with the transit peptide of mitochondrial ATPase 6-subunit (mt-GFP) are shown. Fluorescence levels of leaves at 680 nm (Chlorophyll) and 522 nm (GFP) were monitored separately under a fluorescence microscope. GFP, chlorophyll, and merged fluorescence are shown in the left, middle, and right panels, respectively. The overlay of green (GFP) and red (chlorophyll fluorescence) is indicated as yellow in the merged images. Bar scale = 5 μm.
effect (Fig. 3C, right panel), indicating that CRR4 binds to the putative cis-acting element of ndhD-1 site in a sequence-specific manner. Taken together with the genetic evidence indicating that CRR4 is required for the RNA editing of ndhD-1, we conclude that a PPR protein, CRR4, is a trans-acting factor required for the RNA editing of ndhD-1.

The C residue of ndhD-1 Is Not Essential for CRR4 Binding—A UV cross-linking experiment using the in vitro editing system revealed that a single factor binds both to the cis-acting element and the RNA editing site (35). If the C nucleotide of ndhD-1 also contributes to CRR4 binding, its editing from C to U may influence the binding of CRR4. We next investigated the possibility that CRR4 preferably binds to the pre-edited RNA rather than the post-edited RNA, which carries U at the editing site. In this analysis, the RB3 probe was modified to carry U at the editing site of ndhD-1, so that the sequence corresponds to that of post-edited RNA. As shown in Fig. 4A, the post-edited RB3 probe also interacted with CRR4, indicating that the C nucleotide in the ndhD-1 site is not essential for CRR4 binding. However, the binding affinity was slightly lower when using the post-edited RNA rather than the pre-edited RNA (compare Fig. 3B, lane 3 with Fig. 4A, lane 3). To further estimate the relative binding affinity for the pre-edited and post-edited RNA, a constant low concentration of labeled RNA probe was incubated
FIGURE 3. Determination of the cis-acting element involved in the binding of CRR4 to ndhD mRNA. A, a schematic representation of RNA probes used for the experiments. B, an electrophoretic mobility shift assay was carried out as described under “Experimental Procedures.” The binding of the recombinant CRR4 to the RB3 probe was examined in lanes 1–6. The binding of the recombinant GST to the RB3 probe was examined in lanes 7–9. The concentrations of labeled RB3 probe, recombinant CRR4, and recombinant GST are indicated above each lane. To lower the background of nonspecific RNA binding, yeast tRNA was added as described for Fig. 2C. C, nonlabeled RNAs for competition, which were added in the concentrations indicated above each lane, was preincubated with the recombinant CRR4 (5.0 nM) and yeast tRNA (1.5 nM) for 5 min before the labeled RB3 probe (0.5 nM) was added. The competition experiments between nonlabeled RB3 probe and RB2 probe are shown in the left and right panels, respectively. The positions of protein-RNA complex and free RNA are indicated by C and F, respectively.

FIGURE 4. Minor contribution of the target C nucleotide in the RNA binding of CRR4. A, an electrophoretic mobility shift assay was carried out as described under “Experimental Procedures.” The binding of the recombinant CRR4 to the post-edited RB3 probe was examined in lanes 1–6. The concentrations of labeled post-edited RB3 probe and the recombinant CRR4 are indicated above each lane. To lower the background of nonspecific RNA binding, yeast tRNA was added as described for Fig. 2C. B, an electrophoretic mobility shift assay was carried out using a fixed RNA probe concentration (0.5 nM) with a range of protein concentrations (0, 0.5, 0.8, 1.3, 2.0, 3.2, and 5.0 nM for lanes 1–7, respectively). Binding experiments with pre-edited RB3 probe and post-edited RB3 probe are shown in the top and bottom panels, respectively. Yeast tRNA was added to lower the background of nonspecific RNA binding. C, the graph shows the binding curves of free RNA probe versus CRR4 concentration for pre-edited RB3 and post-edited RB3 probe. The values are the mean ± S.D. of three independent experiments. D, nonlabeled RNAs for competition, which were added in the concentrations indicated above each lane, was preincubated with the recombinant CRR4 (5.0 nM) and yeast tRNA (1.5 nM) for 5 min before the labeled post-edited RB3 probe (0.5 nM) was added. The competition experiments between nonlabeled pre-edited and post-edited RB3 probe are shown in the left and right panels, respectively. The positions of protein-RNA complex and free RNA are indicated by C and F, respectively.
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with a range of protein concentrations. The $K_d$ value is the concentration of protein at which 50% of the RNA probe bound, and was 1.6 and 3.0 nm, for pre- and post-edited RNA, respectively (Fig. 4, B and C). These results indicate that the recombinant CRR4 binds to the pre-edited RNA with a slightly higher affinity than to the post-edited RNA. CRR4 binding to the post-edited RNA was also exposed to competition with the nonlabeled pre- and post-edited RNA (Fig. 4D). The result similarly indicated that the pre-edited RNA has slightly higher activity for recruiting CRR4 than the post-edited RNA (compare Fig. 4D, left panel, lanes 5 and 6, with Fig. 4D, right panel, lanes 5 and 6). Although the C nucleotide of the editing site is not essential for CRR4 binding, CRR4 slightly favors the pre-edited RNA over the post-edited RNA for binding at least at the in vitro assay level.

DISCUSSION

C-to-U RNA editing in plant organelles requires a cis-acting element for site recognition. The putative trans-acting factor binds to this cis-acting element to directly deaminate the target C or to recruit the second trans-acting factor, an unidentified RNA-editing enzyme. However, the molecular identity of the trans-acting factor has up to now been unclear. The breakthrough was the identification of an Arabidopsis crr4 mutant that is specifically impaired with respect to RNA editing of ndhD-1. The CRR4 gene encodes a member of the PPR protein family (21). PPR proteins are believed to be involved in the RNA maturation processes in organelles and to form a large family, especially in higher plants (5, 23). The 466 genes encoding PPR proteins are roughly sufficient to maintain all of the RNA editing events in Arabidopsis. Although the model in which a PPR protein acts as a trans-acting factor was plausible, we could not eliminate the possibility that CRR4 is involved in RNA maturation processes other than RNA editing and that the function of CRR4 is indirectly involved in RNA editing. In this study, we showed that GFP fused with the transit peptide of CRR4 targets to the plastid (Fig. 1B), and CRR4 specifically binds to the 25 nucleotides of the upstream and the 10 nucleotides of the downstream sequences surrounding the editing site of ndhD-1 (Fig. 3). Furthermore, we have shown that the four alleles of crr4 are specifically defective with respect to RNA editing of the ndhD-1 site (21). We conclude that CRR4 is a trans-acting factor required for the RNA editing of ndhD-1 and propose a model in which a PPR protein acts as a site recognition factor for RNA editing in the plastid (Fig. 5).

The 36 nucleotides, including the ndhD-1 site, are sufficient to recruit CRR4 (Fig. 3). The intergenic region between psaC and ndhD includes an inverted repeat, which possibly forms a stem-loop structure, and a potential Shine-Dalgarno-like sequence (Fig. 2B). The former is probably involved in intergenic RNA cleavage, whereas the latter may be involved in ribosome binding. However, these sequences are not required for CRR4 binding, because the 36 nucleotides lack these sequences. This result is consistent with the fact that the intergenic RNA cleavage between psaC and ndhD is not affected in crr4 (21), indicating that recognition of 36 nucleotide sequence by CRR4 is not related to RNA processing. On the other hand, some RNA editing is coupled with RNA processing or translation (34, 36, 37). In some cases, translation might play the role of unwinding an RNA secondary structure to facilitate access of the editing machinery.

The UV cross-linking experiments using in vitro editing system revealed that both labels in the target C site and the cis-acting element covalently bind to an unidentified 56-kDa protein in the psbE editing site (35). In addition, no additional protein was specifically cross-linked with the editing site, implying that the 56-kDa protein itself might carry out the site recognition and the cytidine deamination (35). Thus, these results favor a single-subunit model of RNA editing machinery. Consistent with this result, we also showed that the C nucleotide in the ndhD-1 site is somewhat involved in CRR4 binding (Fig. 4, B and C), although the binding affinity of recombinant CRR4 for pre-edited RB3 probe was only slightly higher than for the post-edited RB3 probe (Fig. 4, B and C). The competition experiment using the pre-edited and the post-edited RNA also showed that the contribution of the target C nucleotide to CRR4 binding was rather minor (Fig. 4D). This observation is consistent with in vivo studies indicating that the cis-acting element of ndhF carrying U at the editing site derived from a transgene leads to reduction of editing efficiency in endogenous ndhF transcripts (18). This result indicates that the C nucleotide at the editing site is not a critical recognition feature for the trans-acting factor. We are not sure whether the slight preference of the pre-edited RNA to the post-edited RNA for the CRR4 binding in vitro has a physiological significance in vivo.

CRR4 belongs to the PLS subfamily (E subclass) in the PPR protein family according to its motif structure (23). CRR4 consists of 11 PPR motifs, an E motif, and an unknown 15-amino acid motif (Fig. 1A). Biochemical analyses of HCF152 have suggested that the PPR motif acts as an RNA-binding motif (29). Several PPR proteins are capable of binding to nucleic acids (23, 38). CRR4 also interacts with the RNA sequence surrounding the editing site of ndhD-1 (Fig. 3), implying that the PPR motifs have a role in RNA recognition. The sequence of the E motif is also related to the PPR motifs (Fig. 1A). However, the sequence of the PPR motifs in the E motif diverges to some degree from the N-terminal PPR motifs (39). The C-terminal unknown 15-amino acid motif is also related to the E and E+ motif and is well conserved in some PPR proteins, including CRR4. The C-terminal region of CRR4 may have a function that is distinct from PPR motifs in the N-terminal region. Another member of the PLS subfamily, CRR2, is involved in the intergenic RNA

![Diagram of Model of RNA editing in the plastid](image-url)
cleavage between rps7 and ndhB (27). CRR2 is a member of the DYW subclass and has all of the motifs present in CRR4, although the 15-amino acid motif is not conserved. It is unlikely that CRR4 includes any possible domain responsible for cytidine deamination. It is therefore necessary to hypothesize that CRR4 includes any possible domain responsible for cytidine deamination. It is therefore necessary to hypothesize another factor responsible for the deamination of the C nucleotide. We propose a two-component model of RNA editing machinery in plastids, which consists of a trans-acting factor of PPR protein and an unidentified RNA-editing enzyme (Fig. 5). This process of RNA editing in plastids appears to be similar to the C-to-U editing in apolipoprotein B mRNA seen in mammals (40), although the molecules involved in the editing are dissimilar.

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REFERENCES

1. Brennicke, A., Marchfelder, A., and Binder, S. (1999) FEMS Microbiol. Rev. 23, 297–316
2. Smith, H. C., Gott, J. M., and Hanson, M. R. (1997) RNA (Cold Spring Harbor) 3, 1105–1123
3. Bock, R. (2000) Biochimie (Paris) 82, 549–557
4. Maier, R. M., Zeltz, P. Kössel, H., Bonnard, G., Gualberto, J. M., and Grienenberger, J. M. (1996) Plant Mol. Biol. 32, 343–365
5. Shikanai, T. (2006) Cell. Mol. Life Sci. 63, 698–708
6. Tsuzuki, T., Wakasugi, T., and Sugiiura, M. (2001) J. Mol. Evol. 53, 327–332
7. Giege, P., and Brennicke, A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 15324–15329
8. Handa, H. (2003) Nucleic Acids Res. 31, 5907–5916
9. Notsu, Y., Masood, S., Nishikawa, T., Kubo, N., Akiduki, G., Nakazono, M., Hirai, A., and Kadowaki, K. (2002) Mol. Genet. Genomics 268, 434–445
10. Bock, R., Kössel, H., and Maliga, P. (1994) EMBO J. 13, 4623–4628
11. Sasaki, Y., Kozaki, A., Ohmori, A., Iguchi, and H., Nagano, Y. (2001) J. Biol. Chem. 276, 3937–3940
12. Zito, F., Kuras, R., Choquet, Y., Kössel, H., and Wollman, F.-A. (1997) Plant Mol. Biol. 33, 79–86
13. Bock, R., Hermann, M., and Kössel, H. (1996) EMBO J. 15, 5052–5059
14. Chaudhuri, S., Carrer, H., and Maliga, P. (1995) EMBO J. 14, 2951–2957
15. Chaudhuri, S., and Maliga, P. (1996) EMBO J. 15, 5958–5964
16. Hirose, T., and Sugiiura, M. (2001) EMBO J. 20, 1144–1152
17. Miyamoto, T., Obokata, J., and Sugiiura, M. (2002) Mol. Cell. Biol. 22, 6726–6734
18. Reed, M. L., Peeters, N. M., and Hanson, M. R. (2001) Nucleic Acids Res. 29, 6948–6952
19. Farré, J.-C., and Araya, A. (2001) Nucleic Acids Res. 29, 2484–2491
20. Takenaka, M., Neuwirt, J., and Brennicke, A. (2004) Nucleic Acids Res. 32, 4137–4144
21. Kotera, E., Tasaka, M., and Shikanai, T. (2005) Nature 433, 326–330
22. Munekage, Y., Hashimoto, M., Miyake, C., Tomizawa, K., Endo, T., Tasaka, M., and Shikanai, T. (2004) Nature 429, 579–582
23. Lurin, C., Andres, C., Aubourg, S., Bellaoui, M., Bitton, F., Bruyere, C., Caboche, M., Debast, C., Gualberto, J., Hoffmann, B., Lecharrn, A., Le Ret, M., Martin-Magne, M. L., Mireau, H., Peeters, N., Renou, J. P., Szurek, B., Taconnat, L., and Small, I. (2004) Plant Cell 16, 2089–2103
24. Small, I. D., and Peeters, N. (2000) Trends Biochem. Sci. 25, 46–47
25. Barkan, A., Walker, M., Nolasco, M., and Johnson, D. (1994) EMBO J. 13, 3170–3181
26. Fisk, D. G., Walker, M. B., and Barkan, A. (1999) EMBO J. 18, 2621–2630
27. Hashimoto, M., Endo, T., Peltier, G., Tasaka, M., and Shikanai, T. (2003) Plant J. 36, 541–549
28. Meierhoff, K., Felder, S., Nakamura, T., Bechtold, N., and Schuster, G. (2003) Plant Cell 15, 1480–1495
29. Nakamura, T., Meierhoff, K., Westhoff, P., and Schuster, G. (2003) Eur. J. Biochem. 270, 4070–4081
30. Yamazaki, H., Tasaka, M., and Shikanai, T. (2004) Plant J. 38, 152–163
31. Bentolland, S., Alfonso, A. A., and Hanson, M. R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 10887–10892
32. Koizuka, N., Imai, R., Fujimoto, H., Hayakawa, T., Kimura, Y., Kohn-Murase, I., Sasai, T., Kawasaki, S., and Imamura, J. (2003) Plant J. 34, 407–415
33. Kazama, T., and Toriyama, K. (2003) FEBS Lett. 544, 99, 102
34. Wang, Z., Zou, Y., Li, X., Zhang, Q., Chen, L., Wu, H., Su, D., Chen, Y., Guo, J., Luo, D., Long, Y., Zhong, Y., and Liu, Y. G. (2006) Plant Cell 18, 676–687
35. Miyamoto, T., Obokata, J., and Sugiiura, M. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 48–52
36. Karcher, D., and Bock, R. (1998) Nucleic Acids Res. 26, 1185–1190
37. Schmitz-Linneweber, C., Tilllich, M., Herrmann, R. G., and Maier, R. M. (2001) EMBO J. 20, 4874–4883
38. Schmitz-Linneweber, C., Williams-Carrier, R., and Barkan, A. (2005) Plant Cell 17, 2791–2804
39. Rivals, E., Bruyere, C., Toffano-Nioche, C., and Lecharn, A. (2006) Plant Physiol. 141, 825–839
40. Wedekind, J. E., Dance, G. S., Sowden, M. P., and Smith, H. C. (2003) Trends Genet. 19, 207–215