Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1498–1502, February 1993
Biochemistry

Characterization of a host protein associated with brome mosaic virus RNA-dependent RNA polymerase

(RNA replication/eukaryotic translation initiation factor 3)

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Communicated by Robert J. Shepherd, November 30, 1992 (received for review September 25, 1992)

ABSTRACT **The association of host proteins with viral RNA replication proteins has been reported for a number of (+)-strand RNA viruses. However, little is known about the identity or function of these host proteins in viral replication. In this paper we report the characterization of a host protein associated with the RNA-dependent RNA polymerase (RdRp) from brome mosaic virus (BMV)-infected barley. A host protein was specifically and proportionally enriched with BMV RdRp activity through several purification steps. This RdRp-associated host protein reacted with an antiserum prepared against wheat germ eukaryotic translation initiation factor 3 (eIF-3). The RdRp-associated host protein, the p41 subunit of wheat germ eIF-3, and an antigenically related protein from rabbit reticulocyte lysates were all found to bind with high affinity and specificity to BMV-encoded protein 2a, which is involved in viral RNA replication. Moreover, addition of wheat germ eIF-3 or the p41 subunit from wheat germ to BMV RdRp gave a specific and reproducible 3-fold stimulation of (−)-strand RNA synthesis *in vitro*. These results suggest that the barley analog of eIF-3 subunit p41, or a closely related protein, associates with BMV RdRp *in vivo* and is involved in BMV RNA replication. This observation and the established role of translation factors in bacteriophage Qβ RdRp suggest that association with translation factors may be a general feature of RNA replication by (+)-strand RNA viruses.**

Brome mosaic virus (BMV) and many other plant and animal (+)-strand RNA viruses share remarkable similarities in RNA replication proteins and other features of genome expression and replication. Based on these similarities these viruses have been grouped into the alphavirus-like superfamily of (+)-strand RNA viruses (1, 2). BMV is the type member of the bromoviruses, a group of (+)-strand RNA plant viruses. The BMV genome is composed of three messenger-sense RNAs, of which RNA1 and RNA2 encode the trans-acting RNA replication proteins 1a (115 kDa) and 2a (100 kDa), respectively (3). The 1a protein contains domains sharing amino acid similarity with viral and cellular helicases (4) and with the nsP1 protein of Sindbis virus, which has methyltransferase activity thought to be involved in capping of viral RNA (5). The 2a protein shares similarity with many RNA virus replication proteins, including the 3D protein of poliovirus and the β subunit of bacteriophage Qβ replicase, which are RNA-dependent RNA polymerases (RdRps; ref. 6). Based on these similarities, 1a and 2a are referred to as the helicase-like and the polymerase-like RNA replication proteins, respectively.

In contrast to accumulating data on the involvement of viral proteins in RNA replication, little is known about the role of host proteins in this process. Among (+)-strand RNA viruses, bacteriophage Qβ RdRp has served as the paradigm in this regard. Qβ RdRp is a complex of the virus-encoded polymerase β subunit and three cellular proteins: ribosomal protein S1 and elongation factors EF-Tu and EF-Ts (7). Ribosomal protein S1 is involved in template recognition and initiation of RNA replication, while the roles of EF-Tu and EF-Ts in Qβ RNA replication remain to be established (7). A fourth host protein of unknown cellular function and weak association with the RdRp holoenzyme affects the balance of Qβ (+)- and (−)-strand synthesis *in vitro* (7). Knowledge of the role of host proteins in RNA replication of (+)-strand RNA viruses of eukaryotes is even more limited. Association of host proteins with viral replication proteins has been reported for poliovirus, turnip yellow mosaic virus, BMV, and the closely related cucumber mosaic virus (8–11). However, the identities of these host proteins have not been established and no direct evidence for their involvement in RNA replication has been presented.

In this paper we report the copurification and specific enrichment of a host protein with BMV RdRp purified from infected barley cells, show that this host protein is a subunit of eukaryotic initiation factor 3 (eIF-3) or a closely related protein, and demonstrate that addition of further eIF-3 or the implicated eIF-3 subunit stimulates BMV (−)-strand RNA synthesis *in vitro*.

MATERIALS AND METHODS

BMV RdRp Preparation. Preparation of DEAE-purified RdRp from BMV-infected barley plants has been described (12). After removal of KCl by passage through a PD-10 gel filtration column (Pharmacia), the DEAE-purified RdRp was loaded onto an S-Sepharose FF (Pharmacia) column (1 × 5 cm) equilibrated in buffer A (10). The column was washed with 15 ml of buffer A. RdRp activity was eluted with a 15-ml linear 0–500 mM KCl gradient in buffer A. One-milliliter fractions were collected and assayed for RdRp activity as described (10).

Preparation of 2a Affinity Resin and Binding Studies. Overexpression and purification of the 2a protein was as described (12, 13), except that solutions were buffered with 50 mM Hepes, pH 7.5. Purified 2a (2.5 mg) was coupled to Affi-Gel 15 affinity matrix (5 ml) in buffer containing 0.3% sodium *N*-lauroylsarcosine (12) according to the instructions of the supplier (Bio-Rad). The 2a resin was extensively washed with 0.1 M glycine/HCl, pH 2.8, to remove noncovalently bound 2a. For binding studies, 100 μl (packed volume) of 2a resin was equilibrated with buffer A containing 250 mM KCl. After

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Abbreviations: BMV, brome mosaic virus; eIF-3, eukaryotic initiation factor 3; RAHP, RdRp-associated host protein; RdRp, RNA-dependent RNA polymerase.

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centrifugation (500 × g for 5 min) and removal of the buffer, 2a resin was resuspended in 200 μl of DEAE-purified RdRp, mixed overnight at 4°C, pelleted by centrifugation, and washed eight times with 1 ml of buffer A containing 250 mM KCl for 5 min at 4°C. The resin was then resuspended in 200 μl of buffer A for RdRp activity assays or in SDS sample buffer (14) for protein analysis.

Purification of Wheat Germ p41 Protein on 2a Resin. Wheat germ extract (250 μl; Boehringer Mannheim) was mixed with 2a resin as described above. The complexity of the starting material necessitated an additional wash with buffer A containing 1 M LiCl and two washes with buffer A containing 2 M KCl to remove traces of other proteins. The bound p41 was then eluted from the 2a resin by resuspending the resin in 500 μl of 10 mM HCl. After incubation the resin was pelleted by centrifugation and the supernatant was removed. The pH of the supernatant was adjusted to pH 8.0.

Preparation of Translation Factors from Wheat Germ. The purification of translation initiation and elongation factors from wheat germ has been described (15). Rabbit antisera to wheat germ initiation and elongation factors and mouse monoclonal antibodies against wheat germ eIF-3 subunits p56, p45, and p36 have been described (16, 17). Rabbit antibodies enriched for reactivity with wheat germ eIF-3 subunit p41 were prepared by affinity purification using the p41 band excised from a nitrocellulose membrane (15).

Protein Analysis. Protein concentrations were determined by the method of Smith *et al.* (18). Electrophoresis, silver staining, Western blotting, and detection of proteins with antisera were performed as described (12, 13, 19). Antisera against BMV nonstructural proteins 1a and 2a have been described (12, 13).

RESULTS

A Host Protein Specifically Copurifies with BMV RdRp Activity. A template-dependent and template-specific BMV RdRp extract was isolated from BMV-infected barley plants by differential centrifugation and solubilization with Triton X-100 (12, 20). The solubilized enzyme was further purified by sequential Sephacryl S-400HR gel filtration and DEAE-Bio-Gel A and S-Sepharose FF ion-exchange chromatography. This procedure resulted in a 206,000-fold increase in the specific RdRp activity of the final S-Sepharose-purified preparation relative to the initial detergent-solubilized RdRp extract (Table 1). The highly purified RdRp preparation synthesizes *in vitro* (−)-strand RNA products on added BMV RNAs. These (−)-strand RNA products anneal to the (+)-strand RNA template RNAs and appear as double-stranded RNAs in native gel electrophoresis (ref. 10; see also Fig. 2*A* below).

Approximately 12 major proteins were detected in the final RdRp preparation after SDS/polyacrylamide gel electrophoresis and silver staining (Fig. 1*A*, lane SS). The predominant proteins in this preparation had mobilities corresponding to proteins of approximately 160, 115, 100, and 45 kDa (arrowheads in Fig. 1*A*). The 115-kDa and 100-kDa proteins were identified by Western blot analysis as virally encoded 1a and 2a, respectively (Fig. 1*B*).

Based on the established role of translation factors in bacteriophage Qβ RdRp, we used rabbit antisera raised against wheat germ translation factors to examine the host proteins present in BMV RdRp preparations. Barley and wheat are closely related grains and these antisera have been shown to cross-react with translation factors of unrelated plant species (21, 22). As expected, all of the tested antisera against wheat germ elongation factors EF-1α and EF-2 and initiation factors eIF-2, eIF-3, eIF-4A, eIF-(iso)4F, eIF-4F, and eIF-coβ reacted with translation factors present in unfractionated barley cell lysates (data not shown). However, when Western blots of RdRp preparations in different stages of purification were incubated with these antisera, only the antiserum against eIF-3 reacted with purified RdRp preparations (Fig. 1*C*). The high selectivity with which the protein reacting with the antiserum against wheat germ eIF-3 copurifies with BMV RdRp is illustrated particularly well in Fig. 1*D*: although EF-1α can constitute up to 5% of the total cellular protein (23), it could not be detected in DEAE- and S-Sepharose-purified RdRp preparations (Fig. 1*D*). Furthermore, the wheat germ eIF-3 antiserum, which recognizes all 10 subunits of the eIF-3 complex, reacted with only one protein, migrating at ≈45 kDa, in the RdRp preparations (Fig. 1*C*). Examination of successive RdRp fractions reveals a striking enrichment of this protein coincident with the increase in specific RdRp activity during purification. These results suggest that a barley protein that specifically copurifies with BMV RdRp is a subunit of eIF-3 or a closely related protein.

Affinity Purification of the RdRp-Associated Host Protein by 2a Resin. The helicase-like BMV 1a protein can be complexed to the polymerase-like 2a protein *in vitro* (12, 13). In attempts to purify 1a from DEAE-purified RdRp preparations by using 2a covalently bound to a resin, we observed that RdRp activity could be bound by the 2a resin (Fig. 2*A*, lane 3). No differences were observed in the products synthesized by input RdRp, unbound RdRp in the supernatant, or RdRp bound to the 2a resin (Fig. 2*A*). Later experiments showed that all RdRp activity could be bound if sufficient 2a resin was added (data not shown). Treatment of 2a resin and RdRp with micrococcal nuclease did not affect RdRp binding, indicating that the binding is not mediated by nucleic acid.

We treated the 2a resin-bound RdRp with SDS and 2-mercaptoethanol to elute noncovalently bound proteins and compared these eluted proteins with those in the input RdRp and the unbound supernatant fraction. Western blots containing equal amounts of protein from each sample were incubated with a mixture of 1a and 2a antisera, revealing a considerable enrichment of 1a and 2a in the 2a resin-bound fraction (Fig. 2*B*). All of the 2a presumably arose from the input RdRp, since 2a resin alone treated with SDS and 2-mercaptoethanol did not release 2a or 2a fragments (data not shown).

When the same blot was stripped of antibodies to 1a and 2a and reincubated with antiserum against wheat germ eIF-3, we observed that, coinciding with the enrichment of 1a and 2a, there was also considerable enrichment of the eIF-3 immunoreactive material in the 2a resin-bound RdRp (Fig. 2*C*). The above Western blot results were consistent with the protein patterns seen on a silver-stained gel of the same samples (Fig. 2*D*). The most prominent high molecular weight proteins in the bound and eluted fraction (Fig. 2*D*, lane 3) comigrated with the Western blot signals for 1a and 2a. The most prominent protein band in the preparation comigrated with the signal observed in the Western blot incubated

Table 1. Purification of RdRp from BMV-infected barley

Step	Protein conc., mg/ml	RdRp activity*, cpm/ml	Specific activity, cpm/mg	Purification, fold
Triton X-100	23.1	3.6×10^6	1.6×10^5	1
Sephacryl S-400HR	0.511	1.3×10^8	2.5×10^8	1,563
DEAE-Bio-Gel A	0.016	0.9×10^8	5.6×10^9	35,000
S-Sepharose FF	0.003	1.0×10^8	3.3×10^{10}	206,000

*Incorporation of [α-^{32}P]CMP into acid-insoluble products was determined as described (10).

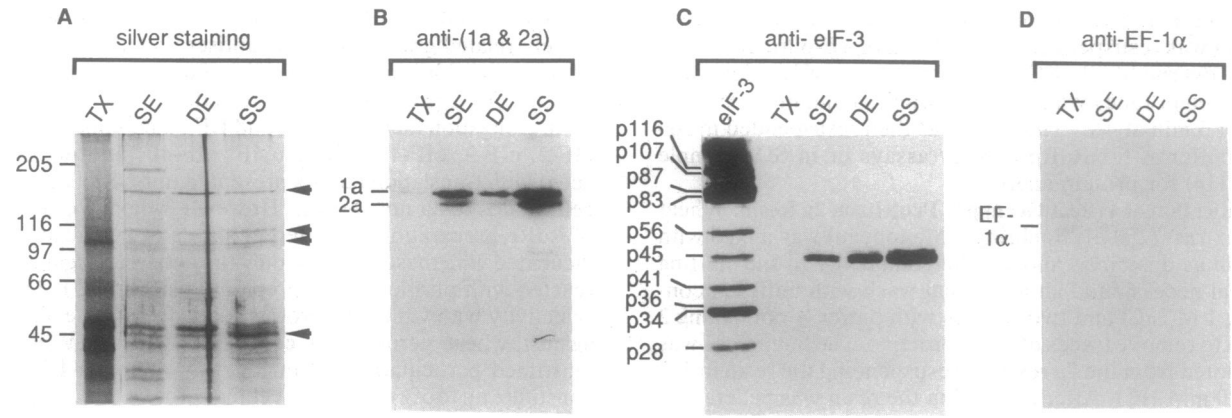

FIG. 1. Copurification and enrichment with BMV RdRp activity of a host protein antigenically related to wheat germ eIF-3. All lanes show 0.5-μg samples of RdRp preparations collected during various stages of RdRp purification. Samples were collected after solubilization of membranes by Triton X-100 (TX) and after subsequent chromatography on Sephacryl S-400HR (SE), DEAE Bio-Gel A (DE), and S-Sepharose FF (SS). The samples were loaded on 10% (*A*) or 12% (*B–D*) polyacrylamide/SDS gels. After electrophoresis, proteins were visualized by silver staining (*A*) or transferred to poly(vinylidene difluoride) (PVDF) membranes (*B–D*) for Western blot analysis and probed with rabbit antisera against 1a and 2a (both diluted 1:10,000) (*B*), eIF-3 (diluted 1:3000) (*C*), or EF-1α (1:10,000) (*D*). A secondary antibody conjugated to horseradish peroxidase (diluted 1:3000) was used with chemiluminescence detection reagents (Amersham) to visualize the antigens. In *A*, the positions and sizes (kDa) of molecular weight markers are indicated on the left and the positions of 160-kDa, 115-kDa, 100-kDa, 45-kDa proteins are indicated by arrowheads on the right. The positions of 1a and 2a are indicated on the left in *B*. Note that the 1a and 2a proteins (middle two arrows in *A*) run at different positions in *A* and *B* due to the different polyacrylamide concentration of the gels used in these experiments. The positions and sizes (kDa) of eIF-3 subunits, and EF-1α are indicated on the left in *C* and *D*, respectively.

with wheat germ eIF-3 antiserum. This comigration was directly confirmed by protein staining of the Western blot used in Fig. 2*C* (data not shown).

Close inspection of the Fig. 2*C* blot and other blots (data not shown) unexpectedly revealed that overnight incubation of the RdRp at 4°C during binding to the 2a resin, or equivalent incubation in the absence of 2a resin, resulted in a small but distinct shift in mobility of the RdRp-associated host protein (RAHP) from 45 kDa (the original mobility in the input RdRp; see Fig. 1*C*) to 41 kDa, comigrating with the p41 subunit of wheat germ eIF-3 (Fig. 2*C*; see also Fig. 3*A*). This coincidental mobility shift might arise from fortuitous proteolysis or, alternatively, from the gain or loss of a charged group or posttranslational modification. Consistent with the latter possibility is the observation that the p41 subunit of wheat germ eIF-3 appears to exist in multiple, electrophoretically distinct isoforms due to presumed posttranslational modification (17). The polyclonal antiserum against wheat germ eIF-3 was enriched for antibodies against the p41 subunit by affinity purification (16). The resulting antiserum reacted with the host protein in BMV RdRp (data not shown). Furthermore, mouse monoclonal antibodies against the p36, p45, and p56 subunits of wheat germ eIF-3 did not react with the RAHP. These results suggest that the RAHP is either the barley analog of the p41 subunit of wheat germ eIF-3 or a closely related protein.

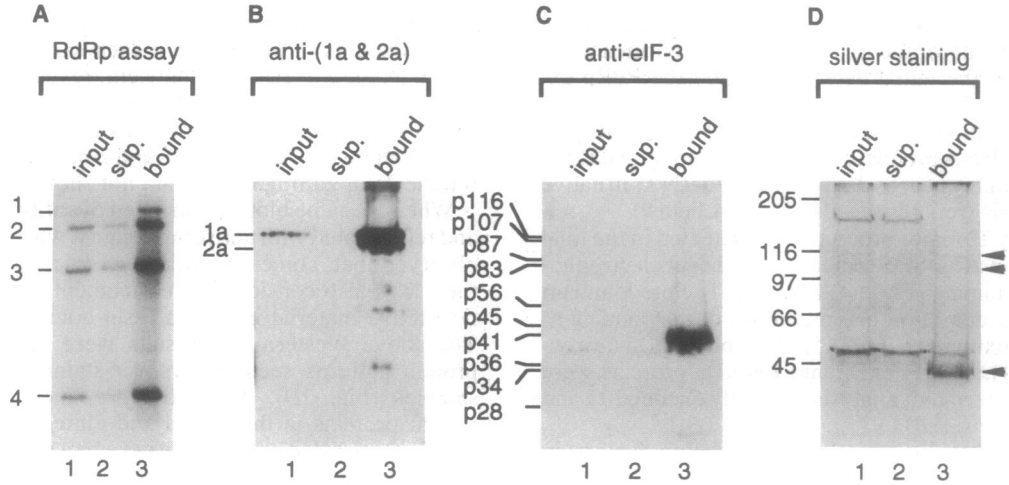

FIG. 2. Binding of BMV RdRp to 2a resin. (*A*) Autoradiograph of double-stranded RNA products synthesized by 50 μl of input DEAE-purified RdRp (lane 1) and by 50 μl of supernatant after pelleting of 2a resin (lane 2). Lane 3 shows the total amount of product synthesized by RdRp bound to 2a resin from 200 μl of DEAE-purified RdRp. After washing, the 2a resin was resuspended in 200 μl of reaction buffer (lane 3). RdRp products were electrophoresed in a 1% agarose gel that was dried and autoradiographed after electrophoresis (10). Positions of double-stranded RNA1–4 are indicated on the left. (*B* and *C*) Identification of RdRp proteins bound to 2a resin. Equal amounts (0.5 μg) of total protein from each sample (see *A* for lane designations) were loaded on a 10% polyacrylamide/SDS gel to show the enrichment of specific proteins after binding. After electrophoresis, proteins were transferred to a PVDF membrane and probed with combined antisera against 1a and 2a (*B*) or antiserum against wheat germ eIF-3 (panel *C*). Positions of 1a and 2a and eIF-3 subunits, respectively, are indicated at left. (*D*) Protein analysis of RdRp proteins bound by and eluted from 2a resin. After electrophoresis as in *B* and *C*, proteins were visualized by silver staining. Positions and sizes (kDa) of molecular weight markers are indicated on the left; positions of stained proteins 1a, 2a, and p41 eluted from RdRp-bound 2a resin are indicated by arrowheads on the right.

Wheat Germ p41 Binds to BMV Protein 2a. Copurification with BMV RdRp of a host protein antigenically related to p41 of wheat germ eIF-3 suggests that this protein interacts with one or more viral replication proteins. Therefore, we tested whether the 2a resin might bind p41 directly from wheat germ extract. The 2a resin was incubated with wheat germ extract, then washed, and bound protein was eluted with SDS/2-mercaptoethanol. Western blotting of the eluted proteins with wheat germ eIF-3 antiserum revealed a single band comigrating with the p41 band of wheat germ eIF-3 and with the RAHP (Fig. 3*A*, lanes 2 and 3). Silver staining of the eluted protein also revealed a single band of this mobility (Fig. 3*B*). Moreover, a comigrating, immunoreactive protein was also bound by 2a resin from an animal translation extract, rabbit reticulocyte lysate (Fig. 3*A*, lane 1). These results support the conclusion of the previous section that the RAHP is a barley analog of the p41 subunit of wheat germ eIF-3 or a closely related protein. No nonspecific protein binding to the 2a resin was observed when the 2a resin was incubated with a mixture of ^{14}C-labeled molecular weight marker proteins (Amersham; data not shown). Also, affinity matrix to which no 2a was coupled but which was otherwise treated identically to the 2a resin did not bind RdRp activity or any protein (data not shown). The binding between 2a and p41 subunit of wheat germ eIF-3 was not disrupted by 2 M KCl/0.1% sodium *N*-lauroylsarcosine or high pH (10 mM Tris·HCl, pH 9; Fig. 3*C*, lanes 1, 3, and 4). Only treatment with 10 mM HCl or with sample buffer containing SDS and 2-mercaptoethanol disrupted the binding (Fig. 3*C*, lanes 2 and 5).

Addition of eIF-3 or p41 to BMV RdRp Stimulates Polymerase Activity. Since the p41 subunit of wheat germ eIF-3 not only shared antigenicity with the RAHP but also bound to 2a resin, we next examined the effect of adding purified wheat germ eIF-3 or 2a-affinity-purified wheat p41 subunit to RdRp (Fig. 3*B*). Despite the abundance of the eIF-3-related host protein already present in the RdRp preparations, a consistent 3-fold stimulation of (−)-strand RNA synthesis *in vitro* was observed in five independent experiments where highly purified eIF-3 complex was added and three experiments where purified p41 subunit was added. Representative results are shown in Fig. 4. Addition of further eIF-3 or p41 did not result in a stimulation greater than that shown in Fig. 4, indicating that saturation was reached.

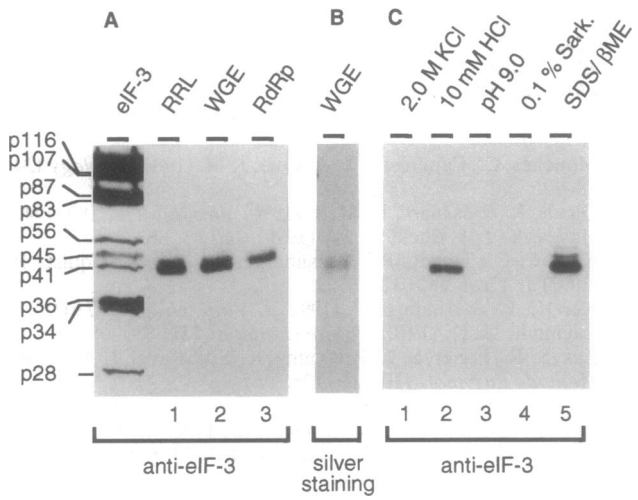

FIG. 3. Interaction of eIF-3 subunit with 2a in the absence of 1a. (*A*) Rabbit reticulocyte lysate (RRL), wheat germ extract (WGE), and RdRp were mixed with separate portions of 2a resin. After the 2a resin was washed, bound proteins were eluted with sample buffer containing SDS and 2-mercaptoethanol, electrophoresed in a 10% polyacrylamide/SDS gel, transferred to PVDF membrane, and incubated with antiserum against wheat germ eIF-3 as described in Fig. 1. (*B*) WGE was incubated with 2a resin and the material eluted as in *A* was electrophoresed in a 10% polyacrylamide/SDS gel. Protein was visualized by silver staining. (*C*) WGE was incubated with 2a resin and, after washing, the resin was divided into several aliquots. Aliquots were treated with either 2 M KCl (lane 1), 10 mM HCl (lane 2), high pH (10 mM Tris·HCl, pH 9.0; lane 3), 0.1% sodium *N*-lauroylsarcosine (sarkosyl) (lane 4), or sample buffer containing SDS and 2-mercaptoethanol (βME; lane 5). Eluted proteins were electrophoresed, transferred to PVDF, and incubated with antiserum against wheat germ eIF-3 as described in Fig. 1. The positions of the subunits of eIF-3 are indicated on the left in *A*.

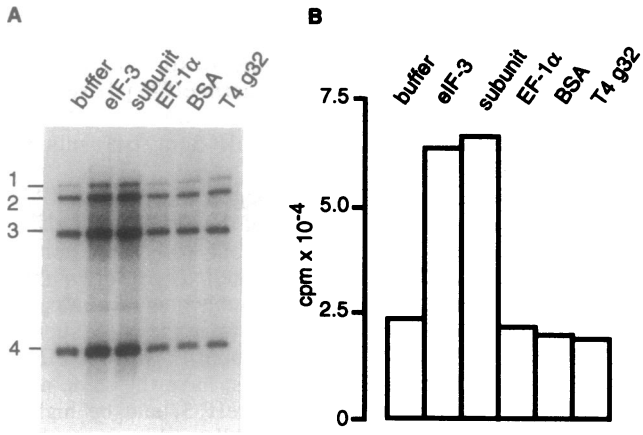

FIG. 4. Stimulation of RdRp activity *in vitro* by wheat germ eIF-3 or the purified eIF-3 p41 subunit. (*A*) Autoradiograph of double-stranded RNA products synthesized *in vitro* by DEAE-Bio-Gel A-purified BMV RdRp samples (0.1 μg) that had been pretreated by overnight incubation at 4°C with either buffer A (10), wheat germ eIF-3 complex (1 μg), purified eIF-3 p41 subunit (10 ng), elongation factor EF-1α (1 μg), bovine serum albumin (BSA, 1 μg), or phage T4 gene *32* protein (T4 g32, 1 μg) as indicated above each lane. Positions of double-stranded RNA1–4 are indicated at left. (*B*) Graph of the results shown in *A*. Radioactivity in double-stranded RNA1–4 on the gel of *A* was determined with a Betascope 603 blot analyzer (Betagen, Waltham, MA).

No significant effect on RdRp activity was observed when RdRp was incubated with buffer, purified wheat germ EF-1α, bovine serum albumin, or bacteriophage T4 gene *32* protein, which binds nucleic acids nonspecifically (Fig. 4). Moreover, the increase in double-stranded RNA products synthesized by RdRp incubated with eIF-3 or p41 was not the result of terminal labeling of the double-stranded RNAs. No labeled products were observed when any of the four nucleoside triphosphates was omitted from the reaction mixture or when unlabeled double-stranded RNA was incubated with eIF-3 or p41 subunit in the absence of RdRp. Also, nuclease S1 treatment did not affect the labeled RNA products (data not shown).

Incubation of RdRp with the antiserum against wheat germ eIF-3 did not inhibit the RdRp activity. However, immunoprecipitation studies showed that these antibodies failed to bind the native RdRp complex (data not shown), indicating that the RAHP is not accessible to the antibodies in the RdRp complex or is not recognized in its native form in the complex. Antibodies against eIF-3 from rabbit reticulocyte lysate also did not have any inhibitory effect on RdRp activity (data not shown). Similarly, although the role of 2a in RNA replication has been established genetically (3), antibodies against 2a do not inhibit BMV RdRp activity *in vitro* (20, 24). Antibodies against 1a do inhibit BMV RdRp *in vitro* (20, 24).

DISCUSSION

In this paper we report the copurification and specific, proportional enrichment of a host protein with BMV RdRp activity. This host protein reacts specifically with antiserum

against wheat germ eIF-3. In addition, both this RAHP and the p41 subunit of wheat germ eIF-3 are bound by the BMV 2a protein, which is a component of the RdRp. After binding to 2a resin, the RAHP and the wheat germ eIF-3 p41 subunit comigrate in SDS/polyacrylamide gel electrophoresis. Moreover, the addition of wheat germ eIF-3 or p41 subunit stimulates RdRp activity.

Together, these results strongly suggest that the RAHP is the barley analog of the p41 subunit of the wheat germ eIF-3 or is a closely related protein. The only other possibility appears to be that the RAHP is related to a protein that consistently copurifies with eIF-3 without being an integral part of this complex. However, if this were the case, such a contaminating protein would have to copurify with the eIF-3 complex through five purification steps, have the same molecular weight as the p41 subunit of eIF-3, and be highly conserved between plant and animal cells and be present in rabbit reticulocyte as well as wheat germ translation extracts (Fig. 3A).

The p41 subunit of wheat germ eIF-3 bound to BMV 2a in the absence of helicase-like 1a protein, the other BMV-encoded RNA replication factor (Fig. 3). This implies that 2a has the ability to bind at least two distinct proteins, 1a (12, 13) and p41. The binding between 2a and p41 was not disrupted by treatment with 2 M KCl, sodium *N*-lauroylsarcosine, or high pH buffer. This contrasts with the binding between 1a and 2a, which is sensitive to KCl concentrations >0.5 M (12).

A relationship between protein biosynthesis and viral replication was proposed when tRNA-like structures were found in many viral RNAs (for a recent review see ref. 25). In addition to structural resemblence, tRNA-like structures in plant viral RNAs also share functional features with their cellular counterparts; i.e., they can be charged with amino acids and adenylylated (25). The first step of BMV replication, (−)-strand RNA synthesis, is directed by signals in the tRNA-like structure at the 3′ terminus of genomic RNAs (3). The tRNA mimicry and the essential role in RNA replication of these structures suggests that some correlation or regulatory interaction may exist between translation and RNA replication on the RNA level. Our data demonstrate an experimental correlation between translation and RNA replication. Addition of wheat germ eIF-3 or p41 to RdRp gave a reproducible 3-fold stimulation of (−)-strand RNA synthesis (Fig. 4). The observed stimulation was specific to eIF-3 since other proteins, including the single-stranded nucleic acid-binding protein of phage T4, did not produce any effect. The inability to further stimulate RdRp activity by further eIF-3 addition might result from near saturation of the starting RdRp preparation with the host protein.

The known functions of eIF-3 in protein synthesis include binding mRNA to the 40S preinitiation complex and stabilization of Met-tRNA binding to the 40S ribosomal subunit (23). The exact role of the eIF-3 complex, much less that of individual subunits, in these steps is not clear. Thus, analyzing the function of the RAHP in BMV RNA replication might also shed some light on the function of this protein in translation. In view of the known eIF-3 functions, it is possible that p41 and its barley analog may be RNA-binding proteins with affinity for RNA templates and rRNA. This would explain why rRNA, but not tRNA, specifically blocks the transcription of viral RNAs by the RdRp of cucumber mosaic virus, a virus closely related to BMV (26). However, other direct and indirect functions of the RAHP can be envisioned. The relatively high abundance of this protein in RdRp preparations, for example, suggests that it might function as a structural protein or scaffold for other RNA replication proteins.

The association of a translation factor or a closely related protein with BMV RdRp and the stimulatory effect of eIF-3 on BMV RdRp are consistent with the established role of translation factors as components of the RdRp of bacteriophage Qβ (7). This suggests that association with translation factors might represent a general feature of RNA replication by (+)-strand RNA viruses. In this respect it is interesting that host proteins with a molecular weight similar to that of the BMV RAHP are associated with the replication proteins of cucumber mosaic virus (11) and turnip mosaic virus (9), which like BMV contain tRNA-like structures at their 3′ termini. It would be interesting to test these host proteins for reactivity with antibodies against eIF-3. Because of the extensive amino acid sequence similarities between the RNA replication proteins encoded by BMV and numerous other plant and animal viruses (1, 2), elucidating the function of the eIF-3-related protein and possibly other RAHPs in BMV RNA replication might have general significance for many other (+)-strand RNA viruses of eukaryotes.

We thank Dr. W. Merrick of Case Western Reserve University for a generous gift of antiserum against rabbit reticulocyte eIF-3, W. De Jong for careful review of the manuscript, and J. M. Ravel, in whose laboratory the wheat germ factors and antibodies were prepared. This research was supported by the National Institutes of Health under Grant GM35072 (P.A.) and the National Science Foundation under Grants DMB9004385 (P.A.) and DMB9105487 (K.S.B.). C.C.K. was supported by National Science Foundation Plant Biology Fellowship DIR-9104366, and R.P.H. was supported by National Institutes of Health Training Grant CA09075.

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