MOLECULAR AND CELLULAR BIOLOGY, Aug. 1992, p. 3390–3398
0270-7306/92/083390-09$02.00/0

Vol. 12, No. 8

The Coat Protein of the Yeast Double-Stranded RNA Virus L-A Attaches Covalently to the Cap Structure of Eukaryotic mRNA

ANTONY BLANC, CHARLES GOYER, AND NAHUM SONENBERG*

Department of Biochemistry and McGill Cancer Center, McGill University, Montreal, Quebec, Canada H3G 1Y6

Received 13 March 1992/Accepted 5 May 1992

The eukaryotic mRNA 5′ cap structure m^7GpppX (where X is any nucleotide) interacts with a number of cellular proteins. Several of these proteins were studied in mammalian, yeast, and drosophila cells and found to be involved in translation initiation. Here we describe a novel cap-binding protein, the coat protein of L-A, a double-stranded RNA virus that is persistently maintained in many *Saccharomyces cerevisiae* strains. The results also suggest that the coat protein of a related double-stranded RNA virus (L-BC) is likewise a cap-binding protein. Strikingly, in contrast to the cellular cap-binding proteins, the interaction between the L-A virus coat protein and the cap structure is through a covalent bond.

All eukaryotic cellular mRNAs (except for organellar mRNAs) possess the 5′ structure m^7GpppX (where X is any nucleotide), termed cap. The cap structure plays important roles in several cytoplasmic and nuclear processes. It is of particular importance in translation, at the initiation level (see references 14 and 51 for reviews). The cap also facilitates mRNA splicing (15, 28) and 3′ end processing (19, 25) and is required for nucleocytoplasmic RNA transport (24). In addition, the cap protects mRNA against 5′-3′ exonucleolytic degradation (18, 21).

Cellular or viral proteins that specifically interact with the cap, cap-binding proteins (CBPs), were identified either by virtue of their specific cross-linking to the cap or by their affinity purification on a cap analog resin. A CBP that acts as a translation initiation factor, eIF-4E, has been isolated and cloned from several species (2, 3, 27, 44). In yeast cells, eIF-4E is encoded by *CDC33* (6). eIF-4E in mammalian and yeast cells is a 24-kDa polypeptide, which associates with one or two other polypeptides to form the CBP complex, eIF-4F (13, 20, 22, 52). eIF-4F is directly involved in cap recognition and allows the interaction of several other initiation factors with the 5′ untranslated region of an mRNA to facilitate binding of the 40S ribosomal subunit (for reviews, see references 31, 33, and 51). Several nuclear CBPs were identified in mammalian cells by using cross-linking techniques (36, 42). One of these proteins, a polypeptide of 80 kDa, was purified from HeLa cells (35). Influenza virus also encodes a CBP (BP-2) that is involved in the capture of cellular mRNA 5′ fragments for use as primers in viral RNA synthesis (5).

Most laboratory strains of *Saccharomyces cerevisiae* harbor one or more of five major double-stranded RNA (dsRNA) virus families (L-A, L-BC, M, T, and W; for reviews, see references 56 and 57). L-A, L-BC, and M represent distinct groups of closely related variants which are found in intracellular particles composed of a single dsRNA genome encapsidated by 60 to 120 coat polypeptide monomers (8, 11). The L-BC genome shares no sequence homology with the L-A genome (49). The sequence of the L-A strain has been determined, and it was shown that the plus strand is 4.5 kb in length and contains two overlapping open reading frames (ORFs) (10, 26). ORF1 encodes the coat protein reported to migrate on sodium dodecyl sulfate (SDS)-polyacrylamide gels as a 81- to 88-kDa polypeptide (16, 49). A fusion of ORF1 and ORF2 resulting from a −1 frameshift generates a 180-kDa Gag-Pol-like protein. This 180-kDa protein exhibits homology to RNA-dependent RNA polymerases (10, 26) and is postulated to have RNA-dependent RNA polymerase activity, which has been shown to be associated with L-A particles in vitro (17, 38). M viruses are satellite viruses requiring both the coat and the fusion proteins from L-A to encapsidate and replicate their smaller genomes (1.8 kb). The M genome encodes a single polypeptide which is processed and secreted as a toxin (the killer toxin), forming lethal pores in the plasma membrane of sensitive cells (for a review, see reference 9). L-BC viruses have smaller coat proteins than do L-A viruses (73 to 77 kDa [49]), which cannot encapsidate M dsRNAs (16).

In a recent report on CBPs in yeast cells, we described a 96-kDa protein (referred to as a 93-kDa polypeptide throughout this report) which is capable of spontaneously cross-linking to capped RNA without UV light induction (20). Here, we identify this polypeptide as the coat protein of the yeast dsRNA virus L-A and characterize its interaction with mRNA cap structures.

MATERIALS AND METHODS

Materials. SP6 polymerase was purchased from Promega. [α-^{32}P]GTP (3,000 Ci/mmol) and [5-^{3}H]CTP (27.1 Ci/mmol) were obtained from Du Pont-New England Nuclear. m^7GMP, m^7GDP, m^7GpppG, GDP, and GpppG were supplied by Pharmacia LKB Biotechnology. Polyethyleneimine-cellulose 3000 UV_{254} thin-layer chromatography plates were from Macherey-Nagel, and the buffers HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), Bicine (*N*,*N*-bis[2-hydroxyethyl]glycine), and CAPS (3-[cyclohexylamino]-1-propanesulfonic acid) were from Sigma. Endoproteinase Lys-C was from Boehringer Mannheim.

Yeast strains, plasmid, and general methods. Yeast strains (Table 1) were grown in YPD medium. Strains 1020, 1773,

* Corresponding author.

TABLE 1. Strains used

Strain	Genotype	dsRNAs
AMR1	*MATa ade2-1 ura3-1 his3-11 leu2-3,112 nat1-5::LEU2 trp1-1 can1-100*	(L-A, L-BC)[a]
EJ101	*MATα trp1 pro1-126 prb1-112 pep4-3 prc1-126*	(L-A, L-BC)[a]
1020	*MATα his4 kar1-1*	L-A-o, L-BC
1773	*MATa arg9*	L-o, M-o
1774	*MATa arg9*	L-A-NH, L-BC-o, M-o
2508	*MATa thr1*	L-A-o, L-BC, M-o

[a] Linkage reactions with extracts from this strain revealed the presence of labeled polypeptides corresponding to the coat proteins of both L-A and L-BC.

1774, and 2508, as well as CsCl-gradient purified L-A virus, were generously provided by R. B. Wickner (National Institutes of Health Bethesda, Md.). The L-A virus was purified as described by Fujimura et al. (17). Strains EJ101 and AMR1 were gifts from M. Rosbash (Brandeis University) and R. Sternglanz (State University of New York), respectively. Preparation of plasmid DNA, DNA restrictions, and agarose gel electrophoresis were performed by standard methods (46).

Yeast extracts. Subcellular fractionation was done as previously described (20). Briefly, cells were harvested and treated with zymolyase, and the resulting spheroplasts were lysed in a hypotonic buffer with a glass Dounce homogenizer. Debris was spun down at 17,000 rpm for 30 min (Sorvall SS34 rotor), and the supernatant was termed S-30. S-30 was centrifuged at 37,000 rpm for 90 min (Beckman 60 Ti rotor), and the resulting supernatant (S-100) was aliquoted and frozen. The pellet was resuspended in buffer A (20 mM HEPES [pH 7.5], 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 7 mM β-mercaptoethanol, 500 mM KCl) at one-fifth of the S-100 volume and stirred overnight on ice. Ribosomes were pelleted by centrifugation at 37,000 rpm for 90 min (60 Ti rotor), and the supernatant (termed the ribosomal high-salt wash [RSW]) was dialyzed for 3 h against buffer B (20 mM HEPES [pH 7.5], 0.2 mM EDTA, 50 mM KCl, 0.5 mM dithiothreitol, 20% glycerol). An abbreviated protocol was also used; in this method, following S-30 centrifugation, KCl was added to 500 mM with gentle stirring on ice. The mixture was then centrifuged for 90 min at 4°C at 37,000 rpm (Beckman 60 Ti rotor). The supernatant was called S-100+RSW, and the ribosomal pellet was resuspended in one-fifth of the S-100+RSW volume (ribosomal fraction). All fractions were made 10% (vol/vol) in glycerol, aliquoted, and frozen at −70°C.

In vitro transcription and capping reactions. Methods were those of Goyer et al. (20). Plasmid pSP64HβglobinΔ6 (29, 43) linearized with *Bam*HI was used as a template for transcription. Unless otherwise specified, cap-labeled RNA refers to m^7GpppG-RNA which is ^{32}P labeled at the α position to the m^7G group.

UV cross-linking and linkage of proteins to cap-labeled RNAs. UV light irradiation was performed essentially as described previously (37), with minor modifications. Briefly, 5×10^4 to 20×10^4 cpm of cap-labeled RNA (1×10^7 to 3×10^7 cpm/μg) was incubated in a total volume of 30 μl in a mixture containing 17 mM HEPES (pH 7.5), 0.5 mM magnesium acetate, 2 mM dithiothreitol, 0.1 mM GTP, 25 mM potassium acetate, and various amounts of yeast extract at 30°C for 10 min. Reaction mixtures were further incubated under conditions described in the figure legends to effect linkage or were irradiated at 254 nm at a distance of 2.5 cm with a 15-W General Electric G15T8 germicidal lamp for 30 to 45 min at 4°C for UV cross-linking. Samples were subsequently treated with 20 μg of RNase A for 30 min at 37°C and resolved on an SDS–12.5% polyacrylamide gel (except for Fig. 7C, for which a 15% polyacrylamide gel was used) (30). Dried gels were exposed against Kodak X-Omat AR5 or Fuji NX film at −70°C for 12 to 48 h. Quantitation of labeled bands was performed by scanning autoradiograms in the linear range of exposure with a Bio Image system (Millipore). Signal intensities are given in arbitrary units.

m^7GDP-agarose affinity chromatography. CBPs were purified as described previously (12, 13), except for the modifications mentioned in the legend to Fig. 2. Buffer A was 100 mM KCl–20 mM HEPES (pH 7.5)–0.2 mM EDTA–10% (vol/vol) glycerol–7 mM β-mercaptoethanol–1 mM phenylmethylsulfonyl fluoride. Purification was performed at room temperature, but all solutions added to the resin were ice cold. Batch chromatography was used, and the polypropylene tubes (the resin sticks minimally to polypropylene) were continuously rotated end over end.

Protein microsequencing. Approximately 1 nmol of native or reduced and alkylated (1) purified 93-kDa CBP was digested in 2 M urea with two successive aliquots of endoproteinase Lys-C (at a 100:1 [wt/wt] ratio of 93-kDa CBP to protease) according to the manufacturer's instructions. CNBr cleavage was performed on approximately 0.7 nmol of the same protein according to the method of Gross (23). The peptides were separated by high-pressure liquid chromatography on an ABI 130 system (Applied Biosystems Inc.) from a reverse-phase RP-300 7-μm C_8 column (Applied Biosystems), using linear gradients (solvent A, 0.1% trifluoroacetic acid in H_2O; solvent B, 70% acetonitrile–0.085% trifluoroacetic acid in H_2O). Purified peptides were collected manually in 1.5-ml Eppendorf tubes and subjected to gas-phase sequencing in a PI 2090 E integrated microsequencing system (Porton Instruments Inc.). Edman degradations were carried out according to standard procedure 40 of the manufacturer. On-line phenylthiohydantoin-amino acid analysis was as recommended by the manufacturer from a reverse-phase Hewlett-Packard AminoQuant column (200 by 2.1 mm), with a flow rate of 0.2 ml/min at 42°C.

Tryptic peptide analysis. Ribosomal fraction (20 μl; approximately 1 mg) from strain EJ101 was incubated with 15×10^6 cpm of cap-labeled RNA, RNase A digested, and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) as described above. Protein was transferred to nitrocellulose, and the RNA-linked protein was located by autoradiography. The radioactive portion of the nitrocellulose was excised and trypsin digested in situ according to the method of Luo et al. (32) except that the membrane was soaked in 0.5% polyvinylpyrrolidone in H_2O (pH adjusted to 7.5 with NH_4OH) and the H_2O wash was replaced by a 50 mM NH_4HCO_3 (pH 8.0) wash. The peptides were electrophoresed in the first dimension and chromatographed in the second dimension as previously described (55).

RESULTS

Incubation of a yeast extract with cap-labeled mRNA resulted in covalent binding (see below) of two polypeptides to the mRNA (Fig. 1, lane 1). Most of the binding occurred to a 93-kDa polypeptide and was completely blocked by the presence of the cap analog m^7GDP, as we have previously reported (20) (this protein was previously termed 96-kDa

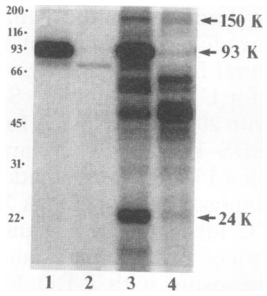

FIG. 1. Spontaneous and UV-induced covalent linkage of proteins in yeast S-100 to mRNA cap structure. Cap-labeled RNA (2×10^5 cpm) was incubated with 100 μg of an S-100 fraction from strain EJ101 for 10 min at 30°C and then either transferred to ice for 1 h (lanes 1 and 2) or irradiated with UV light for 45 min (lanes 3 and 4), in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of 670 μM m^7GDP. Samples were processed for SDS-PAGE and autoradiography as described in Materials and Methods. Positions of molecular size markers (in kilodaltons) are indicated at the left; positions of the CBPs are indicated by arrows at the right.

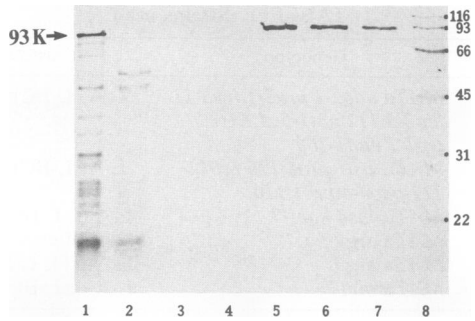

FIG. 2. m^7GDP-agarose affinity chromatography purification of the 93-kDa CBP. Ribosomal fraction (20 ml; approximately 50 mg/ml) from strain EJ101 (lane 1) was incubated for 30 min with 20 ml of m^7GDP-agarose resin. The supernatant (lane 2) was removed after pelleting of the resin at $2,000 \times g$ for 2 min. The resin was washed four times for 5 min each time with 30 ml of buffer A (lane 3, fourth wash) and twice for 5 min with 15 ml 100 μM GDP in buffer A (lane 4, second GDP wash). Three sequential 15-min incubations with 15 ml of 300 μM m^7GDP in buffer A eluted the 93-kDa CBP (lanes 5 to 7). A portion of each fraction (1/1,000) was analyzed by SDS-PAGE. Proteins were visualized by Coomassie blue staining. Lane 8, molecular size markers (indicated in kilodaltons at the right).

polypeptide, but according to the markers used in this study, it migrates with an apparent molecular weight of 93,000). A minor RNA-linked polypeptide migrated as a 75-kDa protein, and its linkage was not affected by the addition of m^7GDP (compare lane 2 with lane 1). Upon UV irradiation, additional polypeptides cross-linked to the mRNA in a cap-specific manner (compare lanes 3 and 4). These polypeptides include the two subunits of the eIF-4F CBP complex, eIF-4E (molecular weight, 24,000) and p150 (molecular weight, 150,000 [20]).

To clone the gene coding for the 93-kDa CBP that binds covalently to the mRNA cap structure, we purified the protein and obtained amino acid sequence information. The starting material from the protease-deficient strain EJ101 was a ribosomal fraction that was enriched in the 93-kDa CBP and deficient in the 75-kDa CBP. The ribosomal fraction is also free of cellular CBPs, which are mostly found in the S-100 fraction. The use of a cap analog-coupled resin (m^7GDP-agarose [12]) offered a convenient one-step purification to near homogeneity. Coomassie blue staining of the fractions collected along the different steps of the purification is shown in Fig. 2. Almost all of the 93-kDa CBP was bound to the affinity column, as no detectable material is seen in the flowthrough fraction (compare lane 2 with lane 1). The 93-kDa protein was not released by GDP (lane 4) but was eluted with excess m^7GDP (lanes 5 to 7). Starting from a wet cell weight of 126 g, the yield of 93-kDa CBP for the purification illustrated in Fig. 2 was 2.4 mg.

Surprisingly, the amino acid sequences of several peptides generated from the purified 93-kDa CBP by cleavage with endoproteinase Lys-C or CNBr were found to have extensive homology with the sequence of the coat protein of the L-A dsRNA virus of *S. cerevisiae* (Fig. 3 [10, 26]). To prove that the 93-kDa CBP was indeed the L-A coat protein, yeast strains that were either free of or harbored L-A or L-BC dsRNA virus were obtained from Reed Wickner (National Institutes of Health). Cell extracts from these strains were incubated with ^{32}P-cap-labeled mRNA, and the resulting RNA-protein complexes are shown in Fig. 4. Extract a was prepared from a strain commonly used in our laboratory (AMR1) that contained both spontaneously RNA-linked polypeptides (lanes 1 and 2). Extract b was prepared from strain 1773 devoid of both L-A and L-BC. No polypeptide in extract b became linked to the cap structure (lanes 3 and 4), even after prolonged incubation times (data not shown), suggesting that both polypeptides are of viral origin. Only the 93-kDa polypeptide bound to mRNA, in a m^7GDP-specific manner (compare lane 6 with lane 5), when an extract containing L-A virus was used (strain 1774; extract c). Finally, a single polypeptide corresponding to the faster-migrating 75-kDa species was linked in extracts prepared from strains 1020 and 2508, both harboring the L-BC virus exclusively (extracts d and e; compare lanes 7 and 9 with lane 1). As observed above (Fig. 1), linkage of the 75-kDa polypeptide was not sensitive to inhibition by m^7GDP (com-

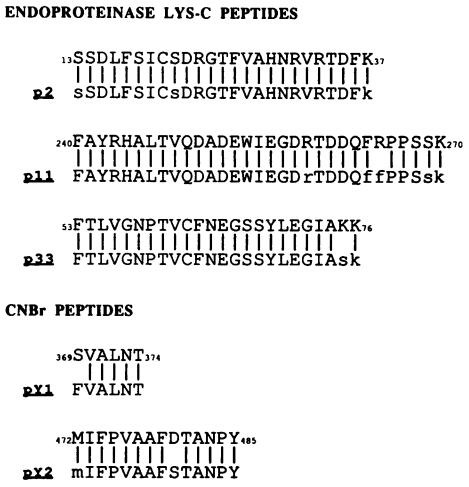

FIG. 3. Alignment of partial amino acid sequences from the 93-kDa CBP and the L-A coat protein. Sequence was obtained by microsequencing peptides isolated from either CNBr (pY1 and pY2)- or endoproteinase Lys-C (p2, p11, and p33)-cleaved 93-kDa CBP. Homologous amino acid sequence from the L-A coat protein (26) is aligned above the sequence of each of these peptides. Residues in lowercase letters denote the most likely amino acids in ambiguous sequencing assignments.

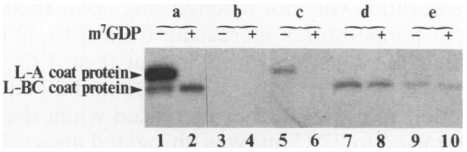

FIG. 4. Linkage of CBPs in extracts from virus-harboring yeast strains. Cap-labeled RNA (10^5 cpm) was incubated in the absence (odd-numbered lanes) or presence (even-numbered lanes) of 670 μM m^7GDP. The following strains were used: a, AMR1; b, 1773 free of L virus (L-o); c, 1774 harboring the L-A virus (L-A and L-BC-o); d and e, 1020 and 2508 harboring the L-BC virus only (L-A-o and L-BC). The following amounts of protein were used: lanes 1 and 2, 100 μg of a 1:1 mixture of S-100+RSW and ribosomal fraction; lanes 3 to 10, 250 μg of S-30 extracts. The samples were processed, electrophoresed, and analyzed by autoradiography as detailed in Materials and Methods.

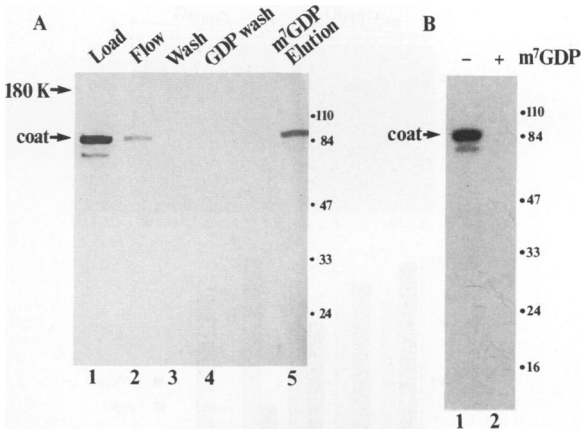

FIG. 5. Cap-binding activity of purified L-A virus. (A) m^7GDP affinity chromatography of L-A virus. L-A virus (about 50 μg) was incubated with 200 μl of the m^7GDP resin (lane 1, load; lane 2, flowthrough). The resin was washed three times (5 min each time) with buffer A (lane 3, third wash) and once (5 min) with 50 μM GDP in buffer A (lane 4). Elution was performed with 300 μM m^7GDP in buffer A for 15 min (lane 5). Ten percent of each fraction was resolved by SDS-PAGE, and the proteins were visualized by Coomassie blue staining. (B) Linkage of purified L-A virus to cap-labeled RNA. Purified L-A virus (10 μg) was incubated with cap-labeled RNA (2×10^5 cpm) for 10 min at 30°C, kept for 45 min on ice, and processed for SDS-PAGE as described in Materials and Methods. The gel was dried and autoradiographed. Lane 1, control; lane 2, in the presence of 670 μM m^7GDP. Positions of molecular size markers (in kilodaltons) are indicated at the right; positions of the coat protein and of the Gag-Pol fusion protein (180 K) are marked by arrows at the left.

pare lane 8 with lane 7 and lane 10 with lane 9). (The stronger signal observed with extract a [compare lanes 1 and 2 with lanes 3 through 10] is due to the use of a mixture of RSW, S-100, and ribosomal fraction that contains larger amounts of coat protein than do the S-30 fractions of the other extracts.) Taken together, these results demonstrate that the 93-kDa polypeptide which is linked to the mRNA cap structure in yeast extracts is the coat protein of the L-A dsRNA virus. Also, these results strongly suggest that the 75-kDa polypeptide is the L-BC coat protein. However, formal proof will require microsequencing of the 75-kDa polypeptide and DNA sequencing of the L-BC genome. (For simplicity, we will hereafter refer to the 75-kDa polypeptide as the L-BC coat protein.) The L-A and L-BC viruses are persistently maintained in most laboratory yeast strains.

To examine the possibility that cellular proteins were involved in mediating covalent bond formation, we assessed the cap-binding activity of purified L-A virus (Fig. 5). The virus was highly pure, as Coomassie blue staining detected only one major band corresponding to the coat protein and two minor bands (Fig. 5A, lane 1). The minor polypeptide below the coat protein is most likely a degradation product of the coat protein, since it reacts with a monoclonal antibody directed against the coat protein on a Western immunoblot (data not shown). The band above the coat protein (indicated as 180 K) is the 180-kDa Gag-Pol-like viral polypeptide. Pure L-A virus was retained on a cap analog resin, as shown by Coomassie blue staining (Fig. 5A; compare lanes 2 and 3 with lane 1). GDP did not elute the protein (lane 4), which was specifically displaced with excess m^7GDP (lane 5). Figure 5B demonstrates that linkage of purified L-A virus to cap-labeled RNA is sensitive to m^7GDP (compare lane 2 with lane 1).

The specificity of the cap-binding activity of the L-A coat protein was further examined. The effects of increasing concentrations of the extended cap analog m^7GpppG, and of its unmethylated counterpart, GpppG, on bond formation are shown in Fig. 6. Linkage was decreased more than 95% in a dose-responsive manner with concentrations of m^7GpppG increasing from 0.67 to 670 μM (Fig. 6A). The nonmethylated cap analog GpppG did not inhibit bond formation between cap-labeled RNA and purified L-A virus (Fig. 6B) (in all lanes of Fig. 6A and B, the lower band is the L-A degradation product mentioned above). Consistent with the absence of inhibition of linkage by GpppG, Fig. 6C demonstrates that GpppG-capped RNA cannot be linked to the L-A coat protein. Quantitation of the binding in Fig. 6A and B is presented in Fig. 6D.

As demonstrated above (Fig. 6), a methylated guanosine is critical for the recognition of the capped structure by the coat protein. To determine the minimal structure required for bond formation, we digested the cap-labeled RNA with RNase A, RNase T_1, nuclease P1, or tobacco acid pyrophosphatase (TAP); the expected products are illustrated in Fig. 7A. Aliquots of each digestion reaction were analyzed by thin-layer chromatography (Fig. 7B). Digestion was complete in all cases, since no label remained at the origin, and the labeled material in lanes 5 and 6 comigrated with the nonradioactive markers m^7GMP and m^7GpppG (compare lane 5 with lane 2 and lane 6 with lane 1). An extract containing both L-A and L-BC viruses was incubated with the various capped oligonucleotides (Fig. 7C). The binding of untreated cap labeled RNA is shown in lane 1. As expected, addition of excess m^7GDP inhibited bond formation to the L-A coat protein (compare lane 2 with lane 1; because an SDS–15% polyacrylamide gel was used, the presumptive L-BC coat protein is not fully resolved from the L-A coat protein). The linkage observed in the presence of m^7GDP (lanes 2 and 4) is most probably due to the L-BC coat protein. RNase A-digested RNA was linked more efficiently than was untreated RNA (compare lane 3 with lane 1 and lane 4 with lane 2). RNase T_1-digested RNA also bound more efficiently than did its untreated counterpart (compare lane 5 with lane 1). The reasons for the enhanced linkage of RNase A and T_1 digestion products will be addressed in Discussion. Neither m^7GpppG nor m^7Gp (the digestion product of nuclease P1 or TAP, respectively) was a substrate for either the L-A or L-BC coat protein (compare lanes 7 and 9 with lane 1 and lanes 8 and 10 with lane 2; a

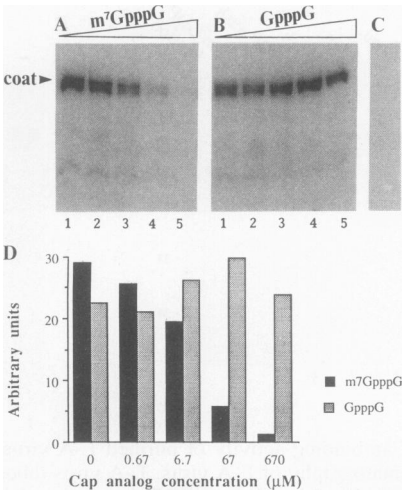

FIG. 6. Effects of cap analogs on linkage of purified L-A virus to m^7GpppG- or GpppG-capped RNAs. (A and B) Linkage to m^7GpppG-capped RNA. L-A virus (10 μg) was incubated for 1 h on ice with m^7GpppG cap-labeled RNA (10^5 cpm) in the presence of 0, 0.6, 6.7, 67, and 670 μM m^7GpppG (panel A, lanes 1 to 5, respectively) or GpppG (panel B, lanes 1 to 5, respectively). (C) Linkage to GpppG-capped RNA. Conditions were as for panels A and B. All samples were processed and electrophoresed as described in Materials and Methods, and the gel was autoradiographed. (D) Quantification of linkage results. The labeled bands in panels A and B were quantified by scanning densitometry, and the resulting values (in arbitrary units) were plotted against cap analog concentration.

longer exposure of the autoradiogram did not reveal any linkage).

We initiated studies to determine the mechanism and site of bond formation on the mRNA. One possibility is that the coat protein catalyzes the cleavage of the mRNA, distal to the cap structure, in conjunction with phosphodiester or phosphoamide bond formation between the cap structure and the protein. Cap-labeled RNA subjected to SDS-PAGE gives rise to an intense smear upon autoradiography, which obscures the signal originating from the RNA-coat protein complex (data not shown). RNase treatment eliminates the smear and allows analysis of the linkage reaction (Fig. 8, lane 1). This treatment, however, makes it impossible to determine whether the RNA is cleaved upon linkage, giving rise to a complex migrating at 93 kDa, or whether the RNA component of the large protein-RNA complex (>250 kDa) is subsequently digested by the nuclease to yield the same 93-kDa complex (we assume that the RNA complex would migrate as a 250-kDa polypeptide [93 kDa + 500 bases of RNA], although we do not have direct evidence for that assumption). Filtering the linkage reactions through nitrocellulose obviates the need for RNase treatment; unbound RNA flows through, while the protein-bound RNA is retained on the filter. The migration of the retained complex (RNase treated or not) was that of a 93-kDa polypeptide (Fig. 8, lanes 2 and 3), suggesting that the RNA is cleaved upon bond formation, possibly at the linkage site.

The conditions for linkage used in the experiments described above were taken from the original report describing this finding (20). To optimize and further characterize the reaction, the effects of time, temperature, and pH on the kinetics of linkage were systematically investigated (Fig. 9). Linkage on ice was linear for long periods of time (up to 32 h), and saturation was not reached (Fig. 9A). Incubations at a range of temperatures increasing from 0 to 30°C demonstrated that the reaction rate, slow at 0 or 4°C, is greatly accelerated at higher temperatures (21 and 30°C; Fig. 9B). The reaction rate was further increased when the temperature was raised to 37°C but was abrogated above 50°C (data not shown). The reaction was initially studied at pH 7.5 (20), but scanning a range of pH values revealed a sharp optimum at pH 8.5 (Fig. 9C). Buffer effects on linkage were minimal, as seen from the smooth transition on the graph from one buffer system to another.

We previously concluded that the interaction between the 93-kDa polypeptide and the cap is covalent on the basis of its stability to SDS-PAGE (20). Figure 10 summarizes our efforts to further examine the stability of this interaction and to confirm its covalent nature. Lane 1 of Fig. 10A illustrates the stability of the interaction to SDS-PAGE. High levels of the cap analog m^7GDP (670 μM; lane 2) and incubation with denaturing reagents such as 8 M urea (lane 3) and 1% SDS (lane 4) at 50°C for 15 min failed to dissociate the formed complex. EDTA (50 mM) did not disrupt the interaction (lane 5), even though the presence of Mg^{2+} is required for the linkage reaction (the same concentration of EDTA inhibits the reaction; lane 7 [20]). The reason for the increase in signal upon addition of EDTA (lane 5) remains unclear. Taken together, these results strongly argue for the formation of a covalent bond between capped RNA and the L-A coat protein. Addition of 50 μM PP_i during the reaction had no effect on the amount of linkage, ruling out a mechanism that was described for cellular and viral capping enzymes, whereby a covalent enzyme-GMP intermediate is generated concomitantly with a release of PP_i (48, 54). To examine the number of sites on the protein at which linkage occurs, RNA-linked L-A coat protein was trypsin digested, and the peptides were subjected to two-dimensional analysis (Fig. 10B). The label was retained on a single peptide which migrated toward the anode, indicating that the linkage occurred to a single site on the coat protein.

DISCUSSION

We describe here a novel covalent interaction between the cap structure of eukaryotic mRNAs and the coat protein of the yeast dsRNA virus L-A and possibly that of the related dsRNA virus L-BC as well. The stability of the chemical bond that is formed between the capped RNA and the L-A coat protein during the linkage reaction is consistent with the formation of a covalent bond. The mechanism of covalent bond formation has yet to be elucidated, but our results suggest that the coat protein engenders the cleavage of the cap structure upon bond formation (Fig. 8). For efficient bond formation to the L-A coat protein, the RNA substrate has to include the 3′ phosphate of the second G in the cap structure (m^7GpppGp*). It is thus likely that this phosphate is directly involved in the reaction.

There are now several instances of covalent linkages between proteins and polynucleotides, and different amino acids have been shown to form the covalent bond. A phosphodiester bond between RNA and tyrosine has been demonstrated for the poliovirus RNA-linked protein VPg (4, 53), and a bond with DNA has been demonstrated for both the bacteriophage φX174 A protein and the gene 52 protein subunit of T4 DNA topoisomerase (41, 47). The tumor suppressor p53 is covalently attached to RNA by a phosphodiester bond to serine (45). If indeed a phosphodiester (or a phosphoamide) bond is formed between the coat protein

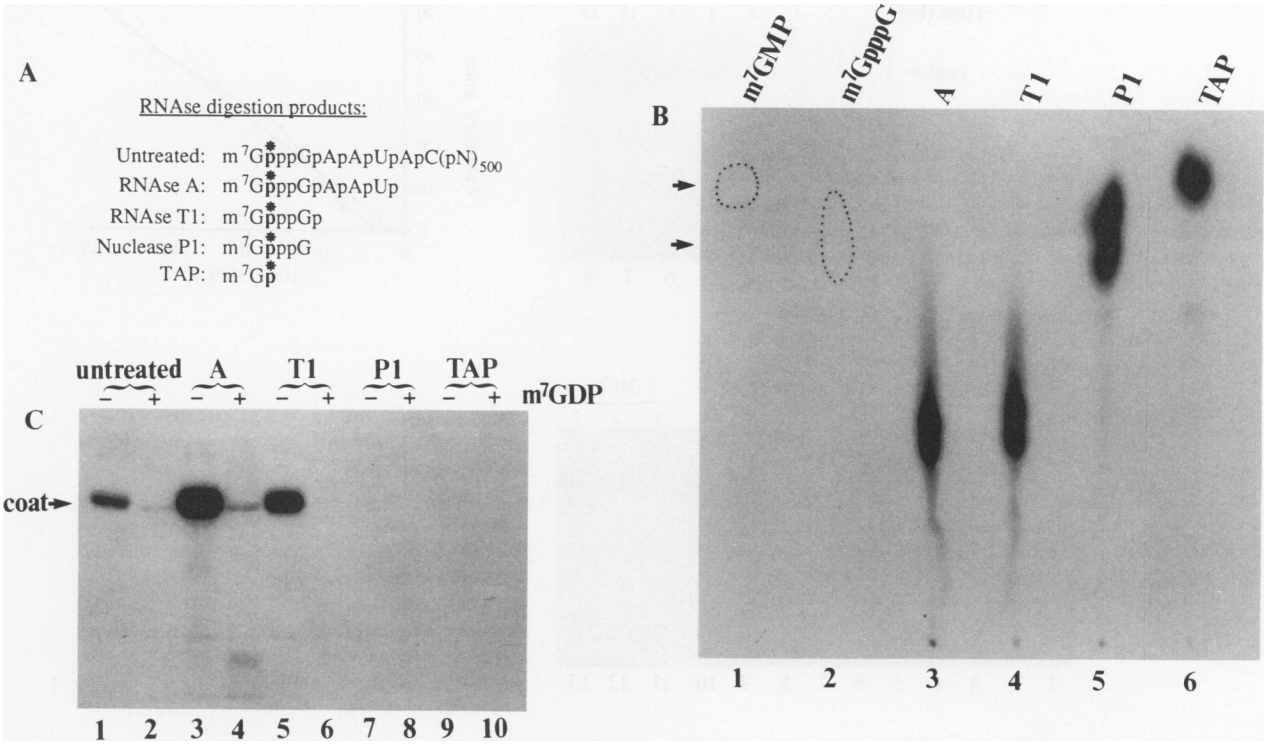

FIG. 7. Minimal size of RNA required for linkage. (A) RNase digestion of cap-labeled RNA. Cap-labeled RNA (2×10^6 cpm; approximately 100 ng) was digested to completion with one of the following nucleases: 50 μg of RNase A (Boehringer Mannheim) in 300 mM NaCl–30 mM Tris (pH 7.5); 5,000 U of RNase T_1 (GIBCO BRL) in 300 mM NaCl–30 mM Tris (pH 7.5)–2 mM EDTA; 20 μg of nuclease P1 (Calbiochem) in 42 mM sodium acetate (pH 5.5); and 10 U of TAP (Sigma) in 50 mM sodium acetate (pH 5.5)–10 mM β-mercaptoethanol–1 mM EDTA. All digestions were for 3 h at 37°C in a volume of 25 to 50 μl. The substrate for TAP was first digested to completion with nuclease P1. The labeled product from each digestion is represented; an asterisk indicates the radiolabeled phosphate. (B) Analysis of digested RNAs by thin-layer chromatography. The digestions were monitored by thin-layer chromatography by spotting aliquots on polyethyleneimine-cellulose 3000 UV_{254} plates that were developed in 1.2 M LiCl. Subsequently, the plates were dried and autoradiographed. Lanes: 1 and 2, standards m^7GMP and m^7GpppG; 3 to 6, approximately 10,000 cpm of RNase A-, RNase T_1-, nuclease P1-, and TAP-digested RNA. (C) Linkage of the RNase-digested cap-labeled RNAs. Each of the RNase digestion products (10^5 cpm) was incubated on ice with 100 μg of ribosomal fraction from strain EJ101 for 90 min. The samples were electrophoresed on an SDS–15% polyacrylamide gel, which was subsequently dried and autoradiographed. m^7GDP (670 μM) was added to all odd-numbered lanes. Lanes 1 and 2 contained undigested RNA; lanes 3 to 10 contained RNAs treated with the indicated nucleases.

and the cap structure, the RNA should be cleaved at the site of the covalent attachment. This view is supported by the finding that in the absence of RNase treatment, the protein-RNA adduct migrated as a 93-kDa polypeptide and not as the larger species expected from the contribution of the RNA. Tobin et al. (53) proposed a model involving RNA cleaving for the self-catalyzed covalent attachment of VPg to poliovirus RNA by a transesterification reaction, which, like the covalent linkage between capped RNA and the L-A coat protein, requires Mg^{2+} and is nucleoside triphosphate independent (20, 53). Similarly, the linkage reaction involving the coat protein could be self-catalyzed, although we have not ruled out catalysis by an enzyme associated with the viral particles. We have demonstrated that the reaction exhibits a sharp pH optimum, suggesting the participation of at least two amino acid residues, one active in the deprotonated form and another active in the protonated form.

The m^7GDP elution of L-A coat protein from a cap analog column argues for a fast and reversible interaction between the cap structure and the L-A coat protein. We propose a two-step mechanism for covalent linkage involving a reversible and very rapid binding of the coat protein to the cap structure followed by a slow and irreversible covalent bond formation at the same active site (while the bond formation

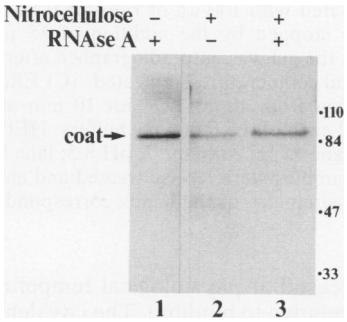

FIG. 8. Cleavage of the cap structure upon linkage. Cap-labeled RNA (10^5 cpm) was incubated with 100 μg of ribosomal fraction from strain 1774 for 4 h at 30°C. RNase A treatment (30 μg for 30 min at 37°C; lanes 1 and 3) was followed by filtration through 0.45-μm-pore-size nitrocellulose filters (lanes 2 and 3). The reaction mixtures were diluted to 1 ml with buffer C (20 mM Bicine [pH 8.5], 2 mM dithiothreitol, 0.5 mM magnesium acetate, 23 mM potassium acetate) prior to filtration. Unbound RNA was washed four times with 1 ml of buffer C, and protein was recovered from the filters by boiling in 3× protein loading buffer. The samples were submitted to SDS-PAGE, and the gel was dried and autoradiographed. Positions of molecular size markers (in kilodaltons) are indicated at the right.

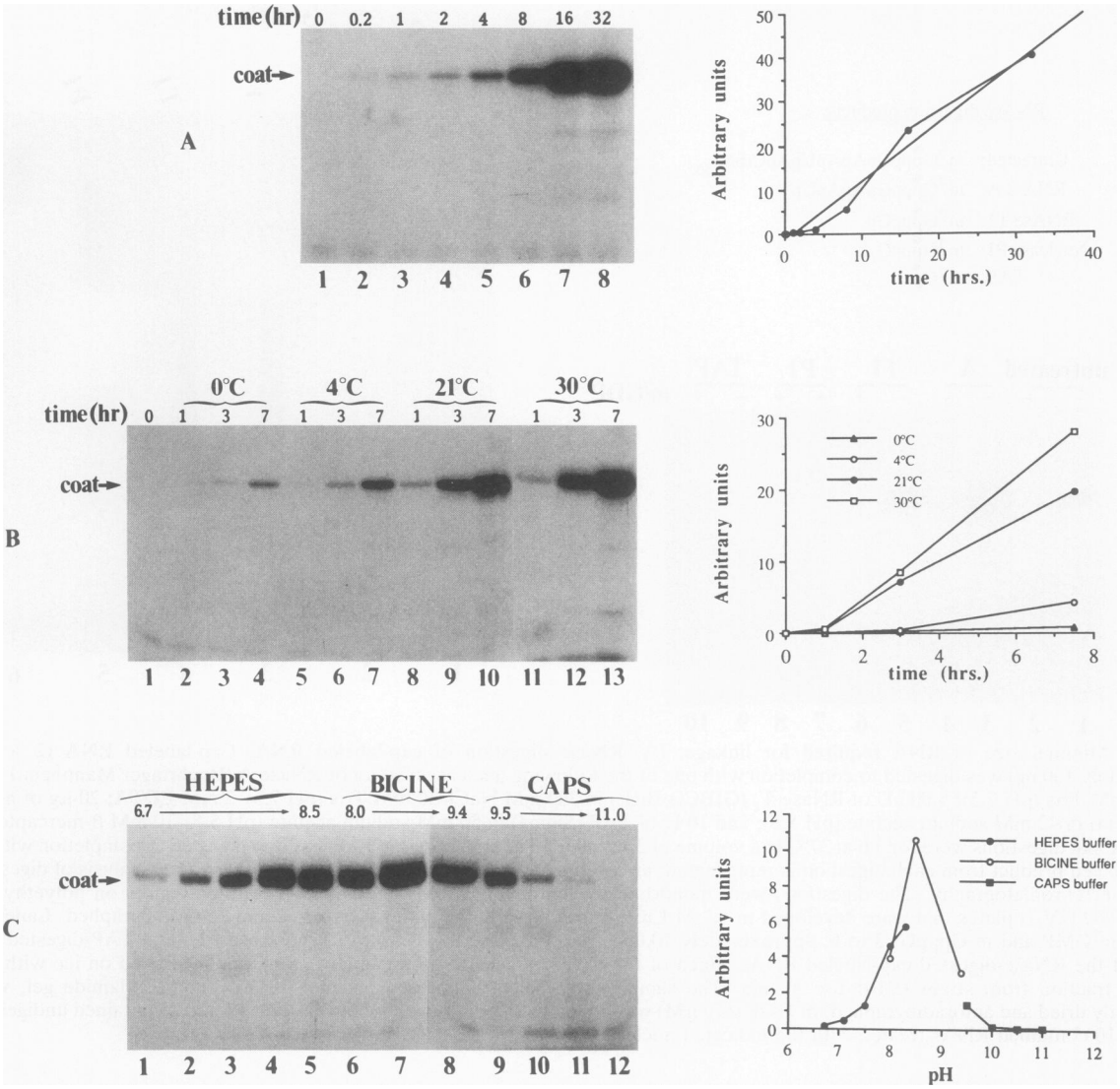

FIG. 9. Time course of the linkage reaction and effects of temperature and pH. (A) Time course. RNase A-digested cap-labeled RNA (10^5 cpm) was incubated with 100 μg of ribosomal fraction from strain 1774 for 10 min at 30°C and transferred to ice for the indicated times. Reactions were stopped by the addition of 15 μl of 3× SDS-PAGE loading buffer, and the samples were stored on dry ice before electrophoresis; the gel was autoradiographed after drying. (B) Effects of temperature on linkage. Conditions were as described for panel A, for the times and temperatures indicated. (C) Effects of pH on linkage. Cap-labeled RNA (2×10^5 cpm) was incubated with 100 μg of ribosomal fraction from strain 1774 for 10 min at 30°C and then for 3 h at room temperature. Reaction mixtures contained a 40 mM concentration of each of the following buffers: HEPES-NaOH (lane 1, pH 6.7; lane 2, pH 7.1; lane 3, pH 7.5; lane 4, pH 8.0; lane 5, pH 8.3), Bicine-NaOH (lane 6, pH 8.0; lane 7, pH 8.5; lane 8, pH 9.4), and CAPS-NaOH (lane 9, pH 9.5; lane 10, pH 10.0; lane 11, pH 10.5; lane 12, pH 11.0). The samples were RNase treated and electrophoresed as described in Materials and Methods. Graphs represent the quantitation by scanning densitometry of the bands corresponding to the L-A coat protein.

is greatly increased at physiological temperatures, the rate remains slow relative to binding). The covalent linkage of the tumor suppressor p53 to RNA was also reported to be a slow reaction (45).

A 5′ N-7-methylated guanosine is probably the minimal requirement common to both binding and bond formation; high concentrations of GpppG do not interfere with linkage of the L-A coat protein, and the virus does not attach to unmethylated capped RNA. The coat proteins of L-A virus and of the presumptive L-BC virus can be affinity purified on m^7GDP-agarose because they recognize and bind to m^7GDP, but they apparently cannot form a covalent bond with this structure. Coat proteins of both L-A and L-BC viruses recognize and form a covalent bond with capped RNA, but only L-A's attachment is inhibited by m^7GDP. This difference might arise from more rigorous structural requirements by the L-BC protein, reflected in the finding that the minimal substrates for efficient linkage to the coat proteins are m^7GpppGp for L-A and the longer m^7GpppGpApApUp for L-BC. The full-length RNA (i.e., not RNase treated) was not bound as well as were the oligonucleotides derived from RNase A or T_1 treatment of the RNA (except for the T_1 product for the L-BC coat protein). This difference might be attributed to the accessibility of the cap structure in the oligonucleotides compared with that in the full-length RNA.

The in vivo significance of the covalent interaction of the

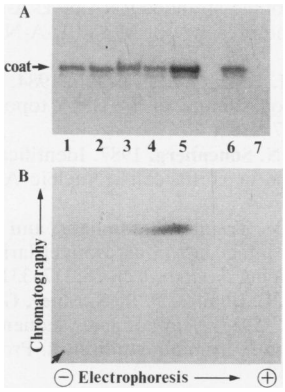

FIG. 10. Chemical nature of the bond between cap-labeled RNA and the L-A coat protein. (A) Stability of the bond. Cap-labeled RNA (2×10^5 cpm) was incubated with 10 μg of L-A virus for 10 min at 30°C and then kept for 1 h on ice. For reactions in lanes 1 to 5, the specified compound was added after the incubation. Lanes: 1, control; 2, 670 μM m^7GDP; 3, 8 M urea; 4, 1% SDS; 5, 50 mM EDTA. For reactions in lanes 6 and 7, 50 μM sodium PP_i (lane 6) and 50 mM EDTA (lane 7) were added to the reaction mixture before the incubation. All reaction mixtures were kept on ice for 1 h, and reaction mixtures in lanes 1 to 5 were further incubated for 15 min at 50°C. (B) Analysis of tryptic peptides obtained from the digestion of RNA-linked L-A coat protein. Analysis was performed as described in Materials and Methods. The arrow indicates the point of sample application. Electrophoresis was in the horizontal direction, and the subsequent chromatography was in the vertical direction.

L-A and L-BC coat proteins with the mRNA cap structure remains an open question. Bruenn and Keitz (7) and Nemeroff and Bruenn (34) demonstrated that neither the viral dsRNA nor the in vitro-produced positive strand was capped at the 5′ end. It is therefore likely that the cap-binding activity is implicated in some interaction with cellular mRNAs. However, the quantitative binding of L-A virus to the cap column suggests that the coat protein is not covalently attached to cap structures in vivo unless only a small fraction of the sites are occupied on each viral particle. One plausible explanation for the apparent absence of covalently attached caps to purified virus is that this interaction is deleterious to the host and therefore is prevented from occurring in the cell. The presence of the known dsRNA yeast viruses does not represent a severe handicap for the host, because the virus copy number (typically 1,000 L-A, 100 L-BC, and 150 M_1 per cell [56]) is curtailed by a class of cellular genes termed *SKI* (superkiller [39, 40, 50, 57, 58]). An intriguing possibility is that the product of one or more of the *SKI* genes prevents the interaction between the virus coat protein and the cap structure of cellular mRNAs.

In summary, we have described a novel and very specific in vitro interaction between the coat protein of L-A virus and the cap structure of cellular mRNAs. Our results also strongly suggest a similar interaction for the coat protein of L-BC virus. Experiments to elucidate the mechanism of covalent bond formation and to investigate the in vivo significance of this phenomenon are currently under way.

ACKNOWLEDGMENTS

We thank Reed Wickner for the virus-harboring yeast strains, for generous amounts of purified L-A virus, and for enlightening discussions. We thank Dave Thomas for valuable comments throughout the course of this work, Aaron Shatkin and Reed Wickner for critically reading the manuscript and Rolf Sternglanz for strain AMR1. We are indebted to Alex Bell and Makoto Yaguchi for invaluable help in obtaining and sequencing peptides. We are grateful to France Dumas for technical help and to Yasuo Konishi for the use of equipment.

This research was supported by a grant from the Medical Research Council of Canada to N.S. A.B. is a recipient of a Medical Research Council of Canada studentship, and C.G. a recipient of a predoctoral research fellowship from the Cancer Research Society (Montreal).

REFERENCES

1. **Allen, G.** 1981. Sequencing of proteins and peptides, p. 30–31. *In* T. S. Work and R. H. Burdon (ed.), Laboratory techniques in biochemistry and molecular biology. Elsevier Science Publishers B.V., Amsterdam.
2. **Altmann, M., I. Edery, N. Sonenberg, and H. Trachsel.** 1985. Purification and characterization of protein initiation factor eIF-4E from the yeast *Saccharomyces cerevisiae*. Biochemistry **24:**6085–6089.
3. **Altmann, M., C. Handschin, and H. Trachsel.** 1987. mRNA cap-binding protein: cloning of the gene encoding protein synthesis initiation factor eIF-4E from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:**998–1003.
4. **Ambros, V., and D. Baltimore.** 1978. Protein is linked to the 5′ end of poliovirus RNA by a phosphodiester linkage to tyrosine. J. Biol. Chem. **253:**5263–5266.
5. **Braam-Markson, J., C. Jaudon, and R. M. Krug.** 1985. Expression of a functional influenza viral cap-recognizing protein by using a bovine papilloma virus vector. Proc. Natl. Acad. Sci. USA **82:**4326–4330.
6. **Brenner, C., N. Nakayama, M. Goebl, K. Tanaka, A. Toh-e, and K. Matsumoto.** 1988. *CDC33* encodes mRNA cap-binding protein eIF-4E of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**3556–3559.
7. **Bruenn, J., and B. Keitz.** 1976. The 5′ ends of yeast killer factor RNAs are pppGp. Nucleic Acids Res. **3:**2427–2436.
8. **Bruenn, J. A.** 1980. Virus-like particles of yeast. Annu. Rev. Microbiol. **34:**49–68.
9. **Bussey, H.** 1991. K1 killer toxin, a pore-forming protein from yeast. Mol. Microbiol. **5:**2339–2342.
10. **Diamond, M. E., J. J. Dowhanick, M. E. Nemeroff, D. F. Pietras, C. Tu, and J. A. Bruenn.** 1989. Overlapping genes in a yeast double-stranded RNA virus. J. Virol. **63:**3983–3990.
11. **Dinman, J. D., T. Icho, and R. B. Wickner.** 1991. A −1 ribosomal frameshift in a double-stranded virus of yeast forms a gag-pol fusion protein. Proc. Natl. Acad. Sci. USA **88:**174–178.
12. **Edery, I., M. Altmann, and N. Sonenberg.** 1988. High level synthesis in *Escherichia coli* of functional eukaryotic initiation factor eIF-4E and affinity purification using a simplified cap-analog resin. Gene (Amsterdam) **74:**517–525.
13. **Edery, I., M. Hümbelin, A. Darveau, K. A. W. Lee, S. Milburn, J. W. B. Hershey, H. Trachsel, and N. Sonenberg.** 1983. Involvement of eukaryotic initiation factor 4A in the cap recognition process. J. Biol. Chem. **258:**11398–11403.
14. **Edery, I., J. Pelletier, and N. Sonenberg.** 1987. Role of eukaryotic messenger RNA cap-binding protein in regulation of translation, p. 335–365. *In* J. Ilan (ed.), Translational regulation of gene expression. Plenum Publishing Corp., New York.
15. **Edery, I., and N. Sonenberg.** 1985. Cap-dependent RNA splicing in a HeLa nuclear extract. Proc. Natl. Acad. Sci. USA **82:**7590–7594.
16. **El-Sheirbeini, M., D. J. Tipper, D. J. Mitchell, and K. A. Bostian.** 1984. Virus-like particle capsid proteins encoded by different L double-stranded RNAs of *Saccharomyces cerevisiae*: their roles in maintenance of M double-stranded killer plasmids. Mol. Cell. Biol. **4:**2818–2827.
17. **Fujimura, T., and R. B. Wickner.** 1988. Replicase of L-A virus-like particles of *Saccharomyces cerevisiae*. *In-vitro* conversion of exogenous L-A and M_1 single-stranded RNAs to double-stranded form. J. Biol. Chem. **263:**454–460.
18. **Furuichi, Y., A. LaFiandra, and A. J. Shatkin.** 1977. 5′ terminal structure and mRNA stability. Nature (London) **266:**235–239.
19. **Georgiev, O., J. Mous, and M. Birnsteil.** 1984. Processing and

nucleo-cytoplasmic transport of histone gene transcripts. Nucleic Acids Res. **12**:8539–8551.
20. **Goyer, C., M. Altmann, H. Trachsel, and N. Sonenberg.** 1989. Identification and characterization of cap-binding proteins from yeast. J. Biol. Chem. **264**:7603–7610.
21. **Green, M. R., T. Maniatis, and D. A. Melton.** 1983. Human β-globin pre-mRNA synthesized *in vitro* is accurately spliced in *Xenopus* oocyte nuclei. Cell **32**:681–694.
22. **Grifo, J. A., S. M. Tahara, M. A. Morgan, A. J. Shatkin, and W. C. Merrick.** 1983. New initiation factor activity required for globin mRNA translation. J. Biol. Chem. **258**:5804–5810.
23. **Gross, E.** 1967. The cyanogen bromide reaction. Methods Enzymol. **11**:238–255.
24. **Hamm, J., and I. W. Mattaj.** 1990. Monomethylated cap structure facilitate RNA export from the nucleus. Cell **63**:109–118.
25. **Hart, R. P., M. A. McDevitt, and J. R. Nevins.** 1985. Poly(A) site cleavage in a HeLa nuclear extract is dependent on downstream sequences. Cell **43**:677–683.
26. **Icho, T., and R. B. Wickner.** 1989. The double stranded RNA genome of yeast virus L-A encodes its own putative RNA polymerase by fusing two open reading frames. J. Biol. Chem. **264**:6716–6723.
27. **Jaramillo, M., J. Pelletier, I. Edery, P. J. Nielsen, and N. Sonenberg.** 1991. Multiple mRNAs encode the murine translation initiation factor eIF-4E. J. Biol. Chem. **266**:10446–10451.
28. **Konarska, M. M., R. A. Padgett, and P. A. Sharp.** 1984. Recognition of cap structure in splicing in vitro of mRNA precursors. Cell **38**:731–736.
29. **Krainer, A. R., T. Maniatis, B. Ruskin, and M. R. Green.** 1984. Normal and mutant human β-globin pre-mRNAs are faithfully and efficiently spliced *in vitro*. Cell **36**:993–1005.
30. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature (London) **227**:680–685.
31. **Linder, P., and A. Prat.** 1990. Baker's yeast, the new work horse in protein synthesis studies: analyzing eukaryotic translation initiation. Bioessays **12**:519–526.
32. **Luo, K., T. R. Hurley, and B. M. Sefton.** 1990. Transfer of proteins to membranes facilitates both cyanogen bromide cleavage and two-dimensional proteolytic mapping. Oncogene **5**:921–923.
33. **Müller, P. P., and H. Trachsel.** 1990. Translation and regulation of translation in the yeast *Saccharomyces cerevisiae*. Eur. J. Biochem. **191**:257–261.
34. **Nemeroff, M., and J. Bruenn.** 1987. Initiation by the yeast viral transcriptase in vitro. J. Biol. Chem. **262**:6785–6787.
35. **Ohno, M., N. Kataoka, and Y. Shimura.** 1990. A nuclear cap binding protein from HeLa cells. Nucleic Acids Res. **18**:6989–6995.
36. **Patzelt, E., D. Blaas, and E. Kuechler.** 1983. Cap binding proteins associated with the nucleus. Nucleic Acids Res. **11**: 5821–5835.
37. **Pelletier, J., and N. Sonenberg.** 1985. Photochemical cross-linking of cap-binding proteins to eukaryotic mRNAs: effect of mRNA 5′ secondary structure. Mol. Cell. Biol. **5**:3222–3230.
38. **Pietras, D. F., M. E. Diamond, and J. A. Bruenn.** 1988. Identification of a putative RNA-dependent RNA polymerase encoded by a yeast double-stranded RNA virus. Nucleic Acids Res. **16**:6226.
39. **Rhee, S.-K., T. Icho, and R. B. Wickner.** 1989. Structure and nuclear localization signal of the SKI3 antiviral protein of *Saccharomyces cerevisiae*. Yeast **5**:149–158.
40. **Ridley, S. P., S. S. Sommer, and R. B. Wickner.** 1984. Superkiller mutations in *Saccharomyces cerevisiae* suppress exclusion of M_2 double-stranded RNA by L-A-NH and confer cold sensitivity in the presence of M and L-A-NH. Mol. Cell. Biol. **4**:761–770.
41. **Rowe, T., K. M. Tewey, and L. F. Liu.** 1984. Identification of the breakage-reunion subunit of T4 DNA topoisomerase. J. Biol. Chem. **259**:9177–9181.
42. **Rozen, F., and N. Sonenberg.** 1987. Identification of nuclear cap specific proteins in HeLa cells. Nucleic Acids Res. **15**:6489–6500.
43. **Ruskin, B., A. R. Krainer, T. Maniatis, and M. R. Green.** 1984. Excision of an intact intron as a novel lariat structure during pre-mRNA splicing *in vitro*. Cell **38**:317–331.
44. **Rychlik, W., L. L. Domier, P. R. Gardner, G. M. Hellmann, and R. E. Rhoads.** 1987. Amino acid sequence of the mRNA cap-binding protein from human tissues. Proc. Natl. Acad. Sci. USA **84**:945–949.
45. **Samad, A., and R. B. Carroll.** 1991. The tumor suppressor p53 is bound to RNA by a stable covalent linkage. Mol. Cell. Biol. **11**:1598–1606.
46. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
47. **Sanhueza, S., and S. Eisenberg.** 1985. Bacteriophage φX174 A protein cleaves single-stranded DNA and binds to it covalently through a tyrosyl-dAMP phosphodiester bond. J. Virol. **53**:695–697.
48. **Shuman, S., and J. Hurwitz.** 1981. Mechanism of mRNA capping by vaccinia virus guanylyltransferase: characterization of an enzyme-guanylate intermediate. Proc. Natl. Acad. Sci. USA **78**:187–191.
49. **Sommer, S. S., and R. B. Wickner.** 1982. Yeast L dsRNA consists of at least three distinct RNAs; evidence that the non-mendelian genes [HOK], [NEX], and [EXL] are on one of these RNAs. Cell **31**:429–441.
50. **Sommer, S. S., and R. B. Wickner.** 1987. Gene disruption indicates that the only essential function of the *SKI8* chromosomal gene is to protect *Saccharomyces cerevisiae* from viral cytopathology. Virology **157**:252–256.
51. **Sonenberg, N.** 1988. Cap-binding proteins of eukaryotic messenger RNA: functions in initiation of translation. Prog. Nucleic Acid Res. Mol. Biol. **35**:173–207.
52. **Tahara, S. M., M. A. Morgan, and A. J. Shatkin.** 1981. Two forms of m^7G-cap binding protein with different effects on capped mRNA translation in extracts of uninfected and poliovirus-infected HeLa cells. J. Biol. Chem. **256**:7691–7694.
53. **Tobin, G. J., D. C. Young, and J. B. Flanegan.** 1989. Self-catalyzed linkage of poliovirus terminal protein VPg to poliovirus RNA. Cell **59**:511–519.
54. **Toyama, R., K. Mizumoto, Y. Nakahara, T. Tatsuno, and Y. Kaziro.** 1983. Mechanism of the mRNA guanylyltransferase reaction: isolation of N^{ϵ}-phospholysine and GMP (5′-N^{ϵ}) lysine from the guanylyl-enzyme intermediate. EMBO J. **2**:2195–2201.
55. **Veillette, A., I. D. Horak, E. M. Horak, M. A. Bookman, and J. B. Bolen.** 1988. Alteration of the lymphocyte-specific protein tyrosine kinase during T-cell activation. Mol. Cell. Biol. **7**:85–96.
56. **Wickner, R. B.** 1986. Double-stranded RNA replication in yeast: the killer system. Annu. Rev. Biochem. **55**:373–395.
57. **Wickner, R. B.** 1989. Yeast virology. FASEB J. **3**:2257–2265.
58. **Wickner, R. B., T. Icho, T. Fujimura, and W. R. Widner.** 1991. Expression of yeast L-A double-stranded RNA virus proteins produces derepressed replication: a *ski*$^-$ phenocopy. J. Virol. **65**:155–161.