Properties of the Avian Viral Protein p12

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SUMMARY

The avian RNA tumour virus structural protein p12 was purified from avian myeloblastosis virus (AMV) by nucleic acid affinity chromatography to apparent homogeneity as judged from SDS-polyacrylamide gel electrophoresis. A filter-binding assay was used for the identification of p12. High concentrations of p12 precipitated nucleic acids out of solution in the absence of MgCl₂. Binding of p12 to single-stranded nucleic acids protected them from digestion with nucleases and resulted in a hyperchromic effect. These phenomena were reversible in the presence of salt. The affinity of p12 to nucleic acids was determined by competing for the binding of p12 to denatured radioactive DNA by various other nucleic acids. It was found that p12 bound preferentially to single-stranded nucleic acids and showed a higher affinity to poly(rI) than to poly(rC) and poly(rA). Purified RNA-dependent DNA polymerase activity from AMV was stimulated up to sixfold by p12, depending on the template. Solubilization of RNA in RNA–DNA hybrids by RNase H was inhibited in the presence of p12.

INTRODUCTION

The gag-region of the genome of avian RNA tumour viruses code for a polyprotein, Pr76gag, which upon processing gives rise to the structural components of the virion p27, p19, p15 and p12 (Eisenman & Vogt, 1978; Vogt et al., 1975). These proteins do not seem to serve solely structural purposes. For example, p15 is also a protease involved in viral polyprotein processing (Collett et al., 1978b; Long et al., 1980; Moelling et al., 1980; van der Helm, 1977), and p19 is highly type-specific and binds preferentially to its homologous RNA at specific sites (Sen & Todaro, 1977). It may play a role in RNA splicing (Leis et al., 1978) in addition to making up the inner virus coat (Bolognesi et al., 1973; Montelaro & Bolognesi, 1978). The p12 is the most basic of the viral structural proteins (Long et al., 1980) and has been found to be tightly associated with the virion RNA (Bolognesi et al., 1973; Fleissner & Tress, 1973). The mechanism of its interaction with the viral RNA has not been characterized. We wanted to investigate its mode of interaction with nucleic acids and furthermore ask whether p12 had any effect on the RNA-dependent DNA polymerase, since RNA-dependent DNA synthesis is known to be inefficient in a reconstituted reaction consisting only of native RNA and enzyme in contrast to virion-associated reactions (Bishop, 1978).

Using disrupted virus particles, optimum conditions for extensive DNA synthesis can be determined (Junghans et al., 1975; Novak et al., 1979). Other virus proteins absent from such a reconstituted system could be responsible for the efficiency of virion-associated DNA synthesis. Indeed, a previous report has described a nucleic acid-binding protein from avian sarcoma virus-transformed cells with unwinding features which appeared to improve in vitro DNA synthesis (Hung & Lee, 1976, 1977). Nucleic acid-unwinding proteins have been
reported in many bacterial and mammalian systems, and appear to be important for DNA replication and genetic recombination (Fleissner & Tress, 1973; Herrick & Alberts, 1975 a, b; Otto et al., 1977; Tsai & Green, 1973; von Hippel & McGhee, 1972). Binding proteins which facilitate RNA conformational changes have not been described extensively. It appeared to us that an RNA-binding protein could serve many functions in the life cycle of the virus, e.g. as a stimulating factor for RNA-dependent DNA synthesis, as a protection against nucleases, as a regulator of protein synthesis and during packaging of the viral genome. This paper describes some properties of p12, indicating that it plays a role in some of these mechanisms.

**METHODS**

**Cells and viruses.** Rous sarcoma virus was grown on chicken embryo fibroblasts as previously described (Owada & Moelling, 1980). Avian myeloblastosis virus (AMV) and disrupted AMV, which had been passed through a DEAE column, was a generous gift of Dr J. Beard, Florida, U.S.A.

**Materials.** Synthetic polynucleotides from *Escherichia coli* and RNA-dependent DNA polymerase originated from Boehringer, Mannheim, native calf thymus DNA type I was obtained from Sigma, and [3H]TTP and [3H]dGTP were bought from New England Nuclear. Selectron filters (BA/85, 0.45 μm) were purchased from Schleicher and Schüll, Dassel, F.R.G.

**Buffers and solutions.** Buffer A: 0.05 M-KH₂PO₄-K₂HPO₄ pH 7.4, 10% glycerol, 1 mM-dithiothreitol (DTT) pH 7.4. TNE: 0.01 M-tris-HCl pH 8, 0.1 M-NaCl, 0.002 M-EDTA. Buffer M: 0.02 M-tris-HCl pH 8, 5% glycerol, 1 mM-EDTA pH 7, 1% dimethyl sulphoxide (DMSO), 5 mM-β-mercaptoethanol.

**Purification of virus, RNA and RNA-dependent DNA polymerase.** AMV was purified by centrifugation through a 20 to 70% sucrose density gradient (Moelling et al., 1979). RNA was extracted by proteinase K, phenol and SDS treatment and the 70S RNA isolated by centrifugation through a 10 to 30% glycerol gradient (Moelling et al., 1979). RNA-dependent DNA polymerase was purified from AMV disrupted with detergent and high salt by column chromatography on a DEAE-cellulose column and subsequently on a phosphocellulose column, both eluted with linear salt gradients (Moelling, 1974; Moelling et al., 1979). The flow-through material of the DEAE-cellulose column was further processed for the isolation of p12.

**Purification of p12.** The DEAE-cellulose column flow-through material was in part supplied by Dr J. Beard, Florida. It was further processed on a DNA agarose column as has been described previously (Moelling et al., 1979). The p12 was eluted in two ways, either with a linear salt gradient ranging from 0 to 0.8 M-KCl in buffer A or alternatively by salt shock elution with 0.8 M-salt in buffer A. This procedure was applied to minimize dilution of p12. The eluted material was dialysed against buffer A and passed through an ultrafiltration membrane (Amicon, UM2OE) with an exclusion limit of about 20000 mol. wt. Pr76 (Pr76 = Pr76*~g, p32 (endonuclease) and residual RNA-dependent DNA polymerase remained in the supernatant. The p12 was analysed for association with DNase, RNase and ATPase activities and fractions containing RNase were not used. The p12 was stored at −20 °C in 0.1% Triton X-100-containing buffer to prevent adherence to the surfaces of the tubes. The final concentration of Triton X-100 in the various assays was lower than 0.01%.

**Preparation of radioactively labelled DNA.** Activated calf thymus DNA was prepared according to Aposhian & Kornberg (1962) using Sigma Type I DNA. A 30 μg amount of this DNA was incubated in a total vol. of 0.5 ml 20 mM-tris-HCl pH 8, 80 mM-NaCl, 8 mM-MgCl₂, 3 mM-DTT 1 mM each of dATP, dGTP, dCTP containing 0.4 μCi ³H-labelled TTP (50 Ci/mmol) and DNA-dependent DNA polymerase from *E. coli*. The mixture was incubated for 2 h at 41 °C, then adjusted to 0.2 M-NaCl, 0.5% SDS and precipitated with 2 vol. of ethanol for 60 min at −70 °C. The pellet recovered after centrifugation was dissolved
in 0.5 ml of 30 mM-sodium acetate, pH 4.5, 0.3 mM-NaCl, 3 mM-ZnCl₂, 0.5% SDS and S1 nuclease (10 units) added before incubation for 2 h at 65 °C. It was then made 2% in SDS and extracted twice with phenol equilibrated with TNE pH 8, once with phenol–chloroform (1:1) and the combined organic phases re-extracted once with TNE. The combined aqueous phases were applied to a Sephadex G-50 column (0.8 × 45 cm), previously equilibrated in TNE pH 8. The DNA was recovered from the exclusion volume and stored at −20 °C.

Filter-binding assay of p12. Membrane filtration assay was performed essentially as described by Davis et al. (1976). Protein samples to be tested for binding ability were suspended in 0.5 ml of membrane buffer (buffer M), before addition of 3H-labelled denatured calf thymus DNA (0.13 μg per assay with approx. 20000 ct/min), which was prepared from the DNA described above by heating and quick cooling. The mixture was then incubated for 5 min at 30 °C. The [3H]DNA–protein complex which formed under these conditions was detected by passing the assay mixture through a membrane filter which was subsequently washed with membrane buffer M. The amount of the radioactively labelled DNA remaining on the filter was then determined by liquid scintillation counting. The filters were pretreated by boiling in distilled water for 20 min. They were subsequently rinsed in cold distilled water and then soaked in membrane buffer M. They could be stored in this buffer for several days.

Competitive membrane filtration assay. To determine the preference of binding of p12 to various nucleic acids, a competitive binding assay was performed, in which increasing amounts of competing unlabelled nucleic acids (5 A₂₆₀/ml corresponding to about 0.2 mg/ml) were added to a filter-binding assay which had been adjusted to a p12 concentration which allowed binding of 50% of the radioactively labelled denatured DNA (0.5 μg p12 and 0.13 μg DNA). Incubation and processing were the same as in the direct assay.

RNA-dependent and DNA-dependent DNA polymerase assays and RNase H test. Standard RNA-dependent and DNA-dependent DNA polymerase assays (Moelling, 1974) contained in 0.1 ml: 0.05 M-tris–HCl pH 7.4, 5 mM-MgCl₂, 2 mM-DTT, [3H]TTP/TTP or [3H]dTGTP/dGTP of specific activity 400 to 4000 ct/min/pmol ([3H]TTP and [3H]dTGTP were 53 Ci/mmol and 15 Ci/mmol respectively). Unlabelled deoxyribonucleotide triphosphates (40 μM each) were present if required. A 5 μg amount of poly(rA).oligo(dT) or poly(rC).oligo(dG) was used as a synthetic template-primer. Alternatively, native 70S RNA (0.5 μg), with or without 0.5 μg oligo(dT) primer or DNA, were used as indicated. Nicked calf thymus DNA was prepared by limited DNase treatment according to Aposhian & Kornberg (1962). One to 2 μl RNA-dependent or DNA-dependent DNA polymerase (0.05 μg/μl) were applied. The effect of p12 was assayed by preincubation of nucleic acid and p12 (0.1 μg/ml) for 10 min at 41 °C in 20 to 30 μl. Subsequently, DNA synthesis was initiated by the addition of the DNA-dependent or RNA-dependent DNA polymerases using the assay conditions mentioned above. Incubation was at 41 °C for 30 min if not stated otherwise. Acid-precipitable radioactivity and RNase H assay was determined as previously described (Moelling, 1974). DNA-dependent DNA polymerase from lymphocytes was a generous gift from Dr B. Otto, Konstanz.

Nucleic acid precipitation assay. Increasing amounts of p12 (0.1 μg/μl) were added to 0.1 μg alkali-denatured 3H-labelled phage λ DNA (10000 ct/min) in a total vol. of 0.1 ml containing: 0.03 M-tris–HCl pH 8.2, 80 mM-KCl, 0.1 mM-EDTA. Incubation was for 16 h at 37 °C. The precipitates were collected by low-speed centrifugation, dissolved in 10 μl 1 M-NaOH and the radioactivity was determined in a scintillation fluid containing toluene and Triton X-100.

RESULTS
Isolation of the viral nucleic acid-binding protein p12
Avian myeloblastosis virus (AMV) was purified, disrupted in the presence of high salt and non-ionic detergent and processed as described for the purification of the viral RNA-
Fig. 1. Isolation of p12. (a) A DNA–agarose column was loaded with AMV proteins present in the
flow-through material of a DEAE-cellulose column (see Methods). Only the nucleic acid-binding
proteins were retained by the DNA–agarose column. Proteins were eluted with 0 to 0.8 M-KCl and an
aliquot (25 μl) of every 5th fraction was analysed on an SDS–polyacrylamide slab gel (7.5 to 20%) and
stained for protein (Dittmar & Moelling, 1978). α and β indicate the two subunits of the
RNA-dependent DNA polymerase (RT), V are virus proteins used as markers. Pr76 indicates the
gag-related polyprotein precursor. (b) Aliquots of each fraction (10 μl) were tested by the filter binding
assay (see Fig. 2 and Methods) for the presence of p12 (●) and for RNA-dependent DNA polymerase
activity using poly(rA).oligo(dT) as a template-primer (○). The ionic strength (A) was determined by
refractive index measurement. (c) Polypeptide analysis of various purification stages of p12. Protein
samples were analysed on a 7.5 to 20% polyacrylamide gel and stained by Coomassie Brilliant Blue.
The polypeptides eluted by salt from the DNA–agarose column were separated by Amicon filtration
into high and low mol. wt. components, the latter containing p12. Some RNA-dependent DNA
polymerase preparations (RT) were found to contain p12 (Moelling et al., 1979).
Avian viral nucleic acid-binding protein

Fig. 2. Interaction of p12 with nucleic acids. (a) Filter-binding assay of p12. A 0.13 μg amount of 3H-labelled denatured calf-thymus DNA (20 000 ct/min) were added to increasing amounts of p12 (0.1 mg/ml) (●) and p15 (0.1 mg/ml) (○) in a reaction mixture containing 500 μl buffer M. After 10 min at 21 °C the amount of each sample was diluted 10-fold with buffer M and passed through a nitrocellulose filter pretreated as described in Methods. After two washes with 5 ml buffer M the filters were dried and radioactivity determined by liquid scintillation counting. (b) Precipitation of nucleic acid by p12. 3H-labelled single-stranded DNA (5000 ct/min in 0.1 μg) was incubated with increasing amounts of p12 in a nucleic acid precipitation assay (see Methods) in the presence of various concentrations of KCl. The precipitates were collected by low-speed centrifugation, dissolved in NaOH and the radioactivity determined. ○, No additional KCl; ●, 50 mM-KCl; △, 100 mM-KCl added during precipitation. (c) Protection of RNA against RNase. 3H-labelled RNA (15 000 ct/min in 0.01 μg) was incubated with p12 (0.1 μg/ml) in a total vol. of 50 μl consisting of 0.1 M-tris-HCl pH 8, 6 mM-MgCl2 and 1 mM-DTT, for 10 min at 4 °C. Subsequently, 0.5 μg/ml RNase A was added for 30 min at 41 °C. Acid-precipitable radioactivity was then determined. (d) Reversion of protection by salt. The assay described in (c) using 1 μg p12 was supplemented by LiCl as indicated and then resistance to nuclease treatment was analysed.

dependent DNA polymerase by column chromatography (Moelling, 1974; Moelling et al., 1979). While most of the RNA-dependent DNA polymerase bound to the DEAE-cellulose column, the structural proteins were recovered from the flow-through material. This material was passed through a single-stranded DNA-agarose column. After washing, the bound proteins were eluted with a linear gradient of 0 to 0.8 M-salt. The polypeptide composition of the fractions was analysed on SDS–polyacrylamide gels and is shown in Fig. 1 (a).

For quantitative analysis of p12 in vitro a filter-binding assay was used. Radioactively labelled denatured DNA was retained on a cellulose nitrate filter in the presence of p12, and
the fractions eluted from the DNA-agarose column were tested with this assay. In parallel, the RNA-dependent DNA polymerase activity was determined. Some of it did not bind to the DEAE-column and co-purified with p12 (Fig. 1b). In a previous study the reverse was observed, some p12 co-purifying with the RNA-dependent DNA polymerase on the phosphocellulose column (Moelling et al., 1979) indicating an association between these proteins.

Alternatively, the column was eluted with 0.6 M-salt to avoid dilution of p12. The eluted material was then fractionated by ultrafiltration through a membrane into high and low mol. wt. components. The high mol. wt. components contained the α and β subunits of the viral RNA-dependent DNA polymerase, a molecule migrating like Pr76 \( ^{\text{Pr76}} \), and a population of molecules with an apparent mol. wt. of 32000, presumably the p32-DNA endonuclease which has been described as a nucleic acid-binding protein (Schiff & Grandgenett, 1978). Fig. 1(c) shows the polypeptide composition of the p12 preparation before and after ultrafiltration and also of the RNA-dependent DNA polymerase which had been prepared by DEAE- and phosphocellulose column chromatography, one preparation contaminated with p12 and the other not (Moelling et al., 1979).

Interaction of p12 with nucleic acids

A sensitive nitrocellulose membrane filtration assay was used for detection of p12. Incubation of radioactively labelled single-stranded DNA in the presence of increasing amounts of p12 resulted in retention of increasing amounts of single-stranded DNA on nitrocellulose filters. A parallel study using increasing amounts of another viral structural protein (p15), which is not known to interact with nucleic acids, gave only a background level of retention (Fig. 2a). Fifty percent of maximum binding was obtained with 0.05 \( \mu \)g p12, which bound about 0.07 \( \mu \)g DNA.

The p12 strongly interacted with single-stranded nucleic acids and was capable of precipitating them out of solution under conditions of low salt and absence of MgCl\(_2\). Such an experiment is shown in Fig. 2(b). Denatured radioactively labelled DNA was treated with increasing amounts of p12 and the amount of precipitated DNA was determined. A 0.3 \( \mu \)g
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1.6

(a) (b)

1.4

1.2

1.0

10 20 30 5 10 15 20

p12 (µg) Time (rain)

Fig. 4. Hyperchromic effect of p12 on nucleic acids. (a) Three µg poly(rI) in a total vol. of 0.5 ml (20 mM-tris-HCl pH 7.4, 5 mM-MgCl₂) were treated with increasing amounts of p12 (0.1 mg/ml). The absorbance at 260 nm was determined at room temperature and corrected for absorption of p12 only. Hyperchromicity was determined as the ratio of absorbance of poly(rI) with and without p12. Addition of 50 µl 10 M-LiCl to the last reaction reversed the hyperchromicity effect (arrow). (b) A 1-5 µg amount of 70S AMV RNA and 15 µg p12 were incubated in 0.5 ml consisting of 0.05 M-tris-HCl pH 8.1, and 0-1 mM-EDTA. The absorbance was determined at the times indicated as described in (a). At time 0 min, p12 was added and at time 20 min, 0.6 M-NaCl was added (arrows).

amount of p12 was required for precipitation of 0.1 µg DNA under the conditions used. Salt ions interfered with the interaction of p12 and nucleic acids; precipitation was inhibited by the addition of 100 mM-KCl (Fig. 2b) or 5 mM-MgCl₂ (data not shown).

If single-stranded RNA was treated with p12 in the presence of MgCl₂ to prevent precipitation, the nucleic acids became resistant towards RNase treatment. 100% resistance was achieved if 0.1 µg nucleic acid was mixed with 1 µg p12 (Fig. 2c). Again, salt interfered with this reaction, 0.1 M-LiCl rendering 75% of the nucleic acid nuclease-sensitive (Fig. 2d).

Specificity of binding of p12 to various nucleic acids

The specificity of interaction of p12 with nucleic acids was analysed using the filter-binding assay described in Fig. 2(a). Radioactively labelled denatured DNA and p12 were adjusted to conditions (0.13 µg DNA and 0.5 µg p12) which allowed binding of 50% of the radioactive DNA to the filter. The extent of competition by increasing amounts of various unlabelled nucleic acids was then measured (Fig. 3). Native DNA and double-stranded homopolymers were poor competitors, while single-stranded RNA competed strongly. Single-stranded synthetic homopolymers showed striking differences in their ability to compete for p12. Poly(rI) was a much more efficient competitor than poly(rC) and poly(rA); 100-fold less poly(rI) was required to give the same competition as poly(rC). The p12 appeared to exhibit a preference for certain polynucleotides, perhaps reflecting preferences for particular base composition or structural features.

Hyperchromic effect of p12 on nucleic acids

The specificity of p12 for single-stranded polynucleotides suggested that it might bind to single-stranded regions of double-stranded nucleic acid complexes, thus lowering the melting temperature of the complex. Disordering of helical structures results in unstacking of the bases and therefore in an increase in light absorption at 260 nm. Such a hyperchromic effect was used to study the interaction between p12 and nucleic acids. When p12 was added to the synthetic homopolymer poly(rI) a hyperchromicity of 1.5-fold resulted (Fig. 4a). A
Fig. 5. Stimulatory effect of p12 on DNA- and RNA-dependent DNA polymerase. A standard RNA-dependent DNA polymerase assay (●) was performed with increasing concentrations of p12 (0.1 mg/ml) using 0.05 μg AMV reverse transcriptase and the following templates: (a) 0.5 μg 70S AMV RNA plus 0.5 μg oligo(dT), (b) 0.5 μg native calf-thymus DNA, (c) 0.5 μg heat-denatured calf-thymus DNA and (d) 0.5 μg DNase-treated calf-thymus DNA. DNA-dependent DNA polymerase α from lymphocytes (○) was tested in parallel. For details of the assays see Methods.

Fig. 6. Kinetics of DNA synthesis in the presence of p12. Fivefold standard reverse transcriptase assays were incubated and five identical aliquots of 100 μl each removed at the indicated times for determination of acid-precipitable radioactivity. 0.05 μg RNA-dependent DNA polymerase, 1 μg p12 were used together with (a) 0.5 μg poly(rC).oligo(dG), (b) 0.5 μg poly(rA).oligo(dT), (c) 0.5 μg heat-denatured calf-thymus DNA and (d) 0.5 μg native calf-thymus DNA. ○, p12 present; ●, p12 absent.
hyperchromic effect was also observed when p12 interacted with 70S viral RNA. Immediately after addition of p12, the absorbance increased 1.15-fold and stayed constant (Fig. 4b). The hyperchromic effects caused by p12 were reversible upon the addition of salt such as 1 M-LiCl (Fig. 4a) and 0.6 M-NaCl (Fig. 4d).

Effect of p12 on RNA-dependent DNA polymerase and RNase H

The binding of p12 to single-stranded nucleic acids, in particular to viral RNA and the hyperchromic effect of p12 on nucleic acids raised the question of whether or not p12 plays a functional role during RNA-dependent DNA synthesis. To analyse this question, transcription of various templates by RNA-dependent DNA polymerase was assayed in the absence and presence of p12. Nucleic acids were pretreated with binding protein p12 and subsequently purified RNA-dependent DNA polymerase was allowed to synthesize DNA. The amount of reverse transcriptase used was determined beforehand to be that allowing 50% of the maximum incorporation. The p12 stimulated DNA synthesis when mixed with 70S viral RNA, denatured or native calf thymus DNA. Stimulation was three- to sixfold, but no stimulation was observed with nicked calf thymus DNA which by itself is a very efficient template (Fig. 5).
In contrast to the other viral nucleic acid-binding protein, p19, which interacts preferentially with the RNA of homologous viral origin, the binding of p12 to nucleic acids was not specific (Sen & Todaro, 1977). We therefore analysed the effect of p12 on another DNA polymerase, the DNA-dependent DNA polymerase α from lymphocytes. DNA polymerase α was stimulated by p12 on activated DNA and on heat-denatured DNA. No stimulation was observed with native DNA or 70S viral RNA, both of which do not normally serve as templates for this enzyme (Fig. 5).

Fig. 6 shows the stimulation of RNA-dependent DNA polymerase by p12 as a function of incubation time. DNA synthesis using poly(rC).oligo(dG) as template was always more susceptible to stimulation by p12 than that using poly(rA).oligo(dT) as template (Fig. 6). This may be because the binding of p12 to poly(rC) was stronger than to poly(dT), as was shown in Fig. 4.

To test the effect of p12 on the actual reverse transcription rather than on DNA-dependent DNA synthesis, native 70S RNA, carrying the natural t-RNA primer, was used as template. A similar stimulation to that described above was observed. Addition of synthetic oligo(dT) primers enhanced RNA-dependent DNA synthesis which was again stimulated by p12 (Fig. 7a, b). Higher concentrations of p12 did not result in further stimulation but rather appeared to be inhibitory (Fig. 7c).

To investigate the effect of p12 on RNase H activity, p12 was incubated with an RNA–DNA hybrid which had been synthesized from φX174 DNA by E. coli RNA polymerase in the presence of 3H-labelled ribonucleotides (Moelling, 1974; Stavrianopolous et al., 1972). Incubation of hybrid and RNase H in the presence of p12 inhibited the release of acid-soluble radioactivity, but no stimulation of RNase H was observed at any concentration of p12 (Fig. 7d).

**DISCUSSION**

The preferential binding of the structural protein p12 from AMV to single-stranded RNA or DNA places it in a category with a number of other nucleic acid-binding proteins. Proteins of this type have been isolated first from T4 phage, the gene 32 protein (Alberts & Frey, 1970) and then also from other organisms including E. coli (Abdel-Monem & Hoffmann-Berling, 1976; Molineux & Gefter, 1974), calf thymus (Herrick & Alberts, 1975a, b), human fibroblasts (Tsai & Green, 1973), mouse ascites (Otto et al., 1977) and others. These proteins are present in cells in great abundance suggesting a structural rather than an enzymic role. They are all involved in DNA replication.

The avian viral structural protein p12 also plays a role during RNA-dependent DNA synthesis. Since about 40 molecules of p12 interact with about 1 molecule of RNA-dependent DNA polymerase (numbers derived from experiment shown in Fig. 5), p12 appears to play a structural role. Its hyperchromic effect on nucleic acids suggests that the stimulatory effect of p12 on RNA dependent DNA synthesis is attributable to the unstacking or unwinding of the nucleic acid. Binding proteins from eukaryotes and prokaryotes are also thought to stimulate the rate of DNA synthesis by extending the DNA (Alberts & Frey, 1970; Herrick & Alberts, 1975a, b). The stimulatory effect of p12 is not specific for RNA-dependent DNA polymerase but also affects mammalian DNA polymerase α and therefore appears less specific than the gene 32 protein from T4, which only acts on its homologous polymerase (Herrick & Alberts, 1975a). The effect of the binding protein p12 is concentration-dependent, and at higher concentrations of p12 the stimulation is reduced (Fig. 7c). The p12 may then prevent interaction of the reverse transcriptase with its template. The enzyme itself exhibits nucleic acid-binding properties (Fig. 1) and perhaps even exerts unwinding functions as well (Collett et al., 1978b). A protein–protein interaction between p12 and reverse transcriptase is
suggested by the extensive co-purification of the two proteins through DEAE-cellulose and phosphocellulose column chromatography (Moelling et al., 1979) and on the DNA agarose column (Fig. 1). It is uncertain, therefore, whether contamination of reverse transcriptase preparations by p12 is responsible for the unwinding effect described by Collett et al. (1978b).

The p12 inhibits RNase H activity (Fig. 7). Since a double-stranded hybrid structure is required for the action of RNase H, opening up of the helix by p12 would be expected to block such an enzyme. The binding protein from E. coli can either inhibit or stimulate exonuclease II, depending on its concentration, while the exonuclease from T4 is inhibited by the gene 32 product (Molineux & Gefter, 1974). Both cases deal, however, with single-strand-specific nucleases in contrast to the RNase H.

A protein exerting a similar effect on RNA-dependent DNA polymerase has been described in chicken cells transformed with avian sarcoma virus (Hung & Lee, 1976, 1977). It is not known whether this protein is identical to p12 or is of cellular origin. It appears that efficient RNA-dependent DNA synthesis does not require cellular factors, since virion-associated RNA-dependent DNA synthesis was found to be very efficient if optimum disruption conditions were applied (Junghans et al., 1975; Novak et al., 1979). This observation suggests the involvement of other viral rather than cellular proteins, in addition to the RNA-dependent DNA polymerase and viral RNA. We have, however, not yet succeeded in demonstrating an effect of p12 on elongation of the initially synthesized 'strong-stop' DNA (cDNA100) in a reconstructed reaction in the presence of p12. Efficient synthesis of a large cDNA in vitro has so far been only obtained with virus (Junghans et al., 1975; Novak et al., 1979) or with the ribonucleoprotein complex (RNP) (Chen et al., 1980) after optimum detergent treatment. It is conceivable that only under conditions of optimum detergent treatment do the native structures remain intact and allow the circularization of the RNA–cDNA100 complex, a prerequisite for synthesis of a large cDNA product involving a hybridization event of the terminally redundant sequences (Collett et al., 1978a; Friedrich & Moelling, 1979; Gilboa et al., 1979; Novak et al., 1979). This event may be facilitated by p12 as well. Preliminary data indicate that p12 increases the renaturation rate of single-stranded nucleic acids (K. W. Sykora & K. Moelling, unpublished observations). Similar properties have been assigned to the nucleic acid-binding protein from E. coli which exhibits catalytic effects on the renaturation of nucleic acids by melting hairpin loops which are normally found in denatured DNA and slow reannealing (Christiansen & Baldwin, 1977).

A nucleic acid-binding protein, p10, has been described in Rauscher murine leukaemia virus. p10 is also a very basic protein, similar to p12 (Long et al., 1980), with preferential binding to single-stranded nucleic acids (Davis et al., 1976; Schulein et al., 1978). The binding observed by Schulein et al. (1978) amounts to about 10 molecules of p10 per 180 nucleotides. Smith & Bailey (1979), who recently analysed p12 from AMV by fluorescence techniques, determined a ratio of about 10 molecules of p12 per 40 nucleotides. In our study it also appears that 10 molecules of p12 bind to 40 nucleotides (Fig. 1). p12 is a basic protein which requires the presence of detergent to stay in solution. On the other hand, detergent reduces the binding of p12 to nucleic acids. It is therefore difficult to derive exact numbers for the p12 to RNA ratio. Smith & Bailey (1979) describe the interaction of p12 with nucleic acids as non-cooperative, an effect not analysed here.

Other interesting biological functions of p12 during the virus life cycle are suggested by some of the experiments reported here; e.g. the resistance of nucleic acids treated with p12 against nucleases may suggest a similarly protective mechanism inside the cell. Furthermore, precipitation of nucleic acids by p12 under certain conditions (Fig. 2) indicates that the RNA can be condensed by p12, a reaction which might be important for RNA packaging during
virion assembly. Even the circular RNP complexes isolated using optimum detergent conditions indicated that the contour lengths are shorter than expected for the RNA genome (Chen et al., 1980).

Several unwinding proteins from mammalian cells have been shown to act on translational mechanisms (Herrick & Alberts, 1975b; Tsai & Green, 1973). It has been reported that the presence of p12 during in vitro translation of viral RNA or the replacement of the RNA by the RNP inhibited protein synthesis (Chen et al., 1980; van der Helm, 1977). We have also observed this phenomenon with a p12 preparation which was free of RNase contamination, and interpret this observation by assuming that p12 prevents translation of the incoming RNA inside the cell and forces the RNA to first undergo reverse transcription. Furthermore, p12 could exert a regulatory effect on translation. Once a certain amount of p12 has been synthesized, further translation may be prevented by feedback inhibition due to the binding of p12 to RNA. This model is complicated by the fact that p12 is synthesized in the form of the polyprotein Pr76

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