Inhibition of the influenza virus RNA-dependent RNA polymerase by antiserum directed against the carboxy-terminal region of the PB2 subunit

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The influenza virus RNA polymerase consists of a heterotrimeric complex of the PB1, PB2 and PA proteins, with the PB2 subunit responsible for recognizing 5' cap structures on the host cell RNAs used as primers for virus mRNA synthesis. To investigate further the role PB2 plays in mRNA synthesis, a set of polyclonal antisera raised against defined regions of the protein were tested for their ability to inhibit the virion transcriptase. All five sera were of sufficient titre to immunoprecipitate PB2 and four were capable of recognizing polymerase complexes containing PB1 and PA. However, only the serum raised against the carboxy-terminus of PB2 (F5) substantially inhibited polymerase activity. This serum drastically reduced synthesis primed by globin mRNA, but only partially inhibited transcription primed by the dinucleotide ApG, or ApG and cap analogue. The preferential inhibition of globin-primed synthesis did not result from interference with cap recognition, as serum F5 did not reduce labelling of PB2 in a photoaffinity cap-binding assay. However, IgG and Fab fragments from F5 were found to inhibit virion endonuclease activity. This suggests that the C terminus of PB2 plays a crucial role in transcription initiation and implicates PB2 in endonuclease activity.

Introduction

The influenza virus genome is transcribed and replicated by a heterotrimeric virus-encoded and packaged RNA-dependent RNA polymerase. On infection of a cell, the three subunits of the polymerase (PB1, PB2 and PA) function in concert with the virus nucleoprotein (NP) to transcribe the virion RNA segments into capped and polyadenylated mRNAs, as well as to replicate the genome via an uncapped and non-polyadenylated (+) strand intermediate (reviewed in Krug et al., 1989). PB1 has been identified as the protein responsible for nucleotide polymerization (Ulmanen et al., 1981; Braam et al., 1983; Romanos & Hay, 1984; Biswas & Nayak, 1994) and it may also act as the backbone of the polymerase complex (Digard et al., 1989). No clear role has been ascribed to PA, although the study of temperature sensitive (ts) mutants has implicated it in RNA replication (Mahy, 1988) and it has been postulated to contain sequence motifs associated with RNA helicase activity (de la Luna et al., 1989). PB2 is associated with one of the most striking features of influenza mRNA synthesis: the use of host cell RNA to prime transcription. PB2 is known to bind to the 5'-cap structures on cellular mRNAs (Ulmanen et al., 1981; Blaas et al., 1982a, b; Penn et al., 1982; Cianci et al., 1995), which are then endonucleolytically cleaved 9–15 bases downstream and the resulting capped RNA fragments used to prime virus transcription (Plotch et al., 1979, 1981; Bouloy et al., 1979, 1980).

Transcription in vitro by the virion-associated polymerase can also be stimulated by the dinucleotide ApG, on the basis of its complementarity to the 3' end of vRNA (Plotch & Krug, 1977; Honda et al., 1986). However, on a molar basis, the dinucleotide is a much less efficient primer than capped mRNAs, suggesting that this mode of priming is artificial and does not play a role in vivo (Plotch et al., 1979). ApG-primed transcription can additionally be stimulated by free cap analogue and as the cap structure is not incorporated into the nascent RNA (unlike ApG), it has been suggested that PB2 can act as an allosteric modulator of the polymerase (Stridh et al., 1981; Penn & Mahy, 1984; Kawakami et al., 1985).
Little is known of the functional regions of the proteins involved in mRNA synthesis, although one study has tested the significance of residues in PB1 implicated (by sequence homology) in polymerase catalysis (Biswas & Nayak, 1994). In addition, the identities of the polymerase component(s) responsible for endonuclease activity remain relatively poorly characterized, although a recently published study has concluded that PB2 is the endonuclease (Shi et al., 1995).

To examine further the role PB2 plays in influenza virus mRNA transcription we tested the ability of specific antisera directed against different regions of the protein to inhibit various enzymatic activities of the virion transcriptase. Here, we show that sera raised against the C terminus of PB2 inhibit the virion transcriptase and that this inhibition results at least partially from inhibition of endonuclease activity.

**Methods**

*Virus and antisera.* Influenza virus strain A/PR8/34 was grown and purified from embryonated eggs according to standard procedures (Barrett & Inglis, 1985). Ribonucleoprotein (RNP) cores were purified through treatment with Triton N-101 and glycerol gradient centrifugation as described by Parvin et al. (1989). The production of antisera reactive against defined regions of the PB2 protein has already been described (Brierley et al., 1987). Briefly, this was accomplished by expressing portions (see Fig. 1) of an A/PR8/34 segment 1 cDNA clone (Young et al., 1983) in *Escherichia coli* as fusion proteins with β-galactosidase and using these polypeptides to immunize rabbits. Similarly produced antisera reactive against PB1 (amino acids 50–370) and PA (amino acids 187–340) have also been described (Digard et al., 1994; Somogyi et al., 1993).

**Immunoglobulin purification.** For studies of *in vitro* transcription and cap structure binding, IgG was purified from the sera by chromatography on DEAE Affi-Gel Blue Sepharose (Hanecak et al., 1982). IgG-containing fractions were pooled and adjusted to a protein concentration of 10 mg/ml. It was established that the preparations still recognized the PB2 protein by performing immunoprecipitations of *in vitro* translated PB2 (data not shown). For the experiments analysing endonuclease activity, IgG was prepared using a MAb Hitrap protein G antibody purification kit (Pharmacia Biotech) using standard protocols. Fab fragments were generated from purified IgG using an Immunopure Fab purification kit (Pierce).

**In vitro transcription of RNA.** For microinjection, capped RNAs encoding the three P proteins were generated by *in vitro* transcription of the appropriate plasmids essentially as described by Digard et al. (1989). Transcription templates were provided by the plasmids pKT1, pKT2 and pKT3 (encoding PB1, PB2 and PA, respectively). These plasmids contain cDNA copies of the specified influenza gene flanked by the 5' and 3' non-coding sequences of the *Xenopus* β-globin gene, under the control of a bacteriophage T7 RNA polymerase promoter (Krieg & Melton, 1984; Digard et al., 1989; Somogyi et al., 1993). RNAs containing isotopically labelled 5'-cap structures for use in endonuclease assays were generated as previously described. In brief, plasmid pGEM-7zf(+) (Promega) was linearized by digestion with *Sal*I and transcribed by bacteriophage SP6 RNA polymerase to generate a 67 nucleotide transcript. The transcript was then 5'-capped and methylated using [α-32P]GTP and vaccinia virus capping enzymes, gel-purified and quantified by spectrophotometry (Chung et al., 1994; Hagen et al., 1994).

**Microinjection of Xenopus oocytes and immunoprecipitation reactions.** Oocytes were taken from frogs, maintained and injected according to standard procedures (Colman, 1984). Oocytes were injected with around 30 ng of mRNA in a volume of 50 nl. After 2 h, the cells were transferred to modified Barth’s saline containing [35S]methionine at 0.5 μCi/ml (5 μCi/oocyte) and incubated overnight. Subsequently, the oocytes were harvested by mechanical disruption into 50 mM-Tris–HCl pH 7.6, 100 mM-KCl, 5 mM-MgCl2, 10% glycerol, 1 mM-DTT and the
extracts clarified by centrifugation. For immunoprecipitation, aliquots (5 µL containing the equivalent of half an oocyte) were diluted into 100 µL of high detergent immunoprecipitation (IP) buffer (50 mM-Tris Cl pH 7.6, 100 mM-KCl, 5 mM-MgCl₂, 1 mM-DTT, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) and left on ice for 30 min before the addition of up to 4 µL antisera. After a further 1 h on ice, 50 µL of a 50% (v/v) suspension of Protein A-Sepharose (Sigma) in high detergent IP buffer was added, and the samples incubated with gentle mixing for 30 min at room temperature. Sepharose-bound material was collected by centrifugation, washed twice with 750 µL of 100 µL of high detergent IP buffer, once with a similar volume of low detergent material was collected by centrifugation, washed twice with 750 µL of 1 mM-DTT, 0.2% NP40 and approximately 0.3 µM-photo-reactive cap analogue [γ-³²P]H-[benzoylphenylamido]-7-methylguanosine-5'-triphosphate (generously provided by C. Penn) supplemented in some cases with 5 µg/ml of purified IgG were incubated for 10 min prior to the addition of endonuclease cocktail containing the radiolabelled in vitro capped RNA (Chung et al., 1994; Hagen et al., 1994). Samples were incubated for 60 min at 31°C before the reaction was terminated by the addition of formamide stop solution, and the products analysed by electrophoresis on 20% polyacrylamide gels containing 7 M-urea. In some experiments, cleaved product was quantified through the use of a Betascope 603 blot analyser (Betagen).

**Transcriptase cap-binding assay.** Quartz cuvettes containing detergent-disrupted virus (15 µg) in a 25 µL reaction volume containing 100 mM-KCl, 15 mM-NaCl, 5 mM-MgCl₂, 50 mM-HEPES pH 7.8, 1 mM-DTT, 0.2% NP40 and approximately 0.3 µM-pho-reactive cap analogue [γ-³²P]H-[benzoylphenylamido]-7-methylguanosine-5'-triphosphate (generously provided by C. Penn) supplemented in some cases with 5 µg/ml of purified IgG were incubated for 10 min at 30°C before irradiation at 320 nm for 5 min at 4°C. The irradiation source was a 500 W high pressure mercury lamp (Philips, Netherlands), focused through a 300 nm filter (Schott and Gen, Germany). The focal point was at 7 cm, with an irradiation of 98 mW/cm². Protein was then concentrated by acetone precipitation before analysis by denaturing polyacrylamide gel electrophoresis and autoradiography.

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**Fig. 2.** Ability of the sera to immunoprecipitate polymerase complexes. Radiolabelled extracts from *Xenopus* oocytes microinjected with synthetic mRNAs encoding (a) PB2, (b) all three P proteins, (c) PB1 or (d) PA were immunoprecipitated with the indicated antisera. Precipitated polypeptides were separated by SDS-PAGE and detected by autoradiography. The migration positions of PB1, PB2 and PA are indicated in (b).

**Results**

**Recognition of polymerase complexes by the sera**

Previously, we had prepared rabbit polyclonal antisera against five overlapping regions of the influenza A/PR8/34 segment 1 product (Fig. 1) expressed in *Escherichia coli* as fusion proteins with β-galactosidase (Brierley et al., 1987). These antisera (but not preimmune bleeds from the corresponding animals) all reacted in immunoprecipitation reactions with in vitro translated PB2 from rabbit reticulocyte lysate (Brierley et al., 1987) and in immunoblots with PB2 from purified virions (data not shown). However, for the region-specific sera to be useful probes of transcriptase function, it was necessary to establish whether they reacted with PB2 in the context of a polymerase complex. We therefore tested the ability of the sera to immunoprecipitate either PB2 alone, or PB2 co-expressed with PB1 and PA. To provide suitable protein targets for immunoprecipitation, extracts were prepared from *Xenopus* oocytes that had been microinjected with in vitro transcribed synthetic mRNAs encoding the three P proteins and metabolically labelled with [³⁵S]methionine. Previous work has shown that as in virus-infected cells (Detjen et al., 1987), all three P proteins associate into a complex in oocytes (Digard et al., 1989). However, the ability to express individual P proteins in oocytes provides a means of assessing the
specificity of the immunoprecipitations, which would not be possible if infected cell-derived material was used.

As expected, all five anti-PB2 sera were able to immunoprecipitate PB2 alone, albeit with varying efficiencies, while a control serum (anti-PB1) was not (Fig 2a). This is in agreement with the ability of all five sera to immunoprecipitate in vitro translated PB2 (Brierley et al., 1987). Previously, we have shown that the F5 anti-PB2 serum was able to immunoprecipitate complexes of all three P proteins from oocytes (Digard et al., 1989) and this finding was replicated (Fig. 2b, lane 6). Three of the other anti-PB2 sera also recognized polymerase complexes, as evidenced by the similar amounts of directly precipitated PB2 and indirectly precipitated PB1 and PA (Fig. 2b; lanes 3–6, sera F2–F4, respectively). However, the amounts of PB1 and especially PA co-precipitated by the F1 serum were reproducibly lower than with the other four anti-PB2 sera (Fig. 2b, lane 2; compare the ratio of PB2 to PB1 and PA with that in lane 5 for example). None of the five anti-PB2 sera reacted significantly with PB1 or PA in the absence of PB2 (Fig. 2c and d), indicating that any co-precipitation of PB1 and PA did not result from lack of specificity of the sera, but was instead a reflection of the protein–protein interactions between the polypeptides.

We therefore concluded that four of the five anti-PB2 sera reacted with PB2 in the form of polymerase complexes, while the F1 serum was either less able to recognize PB2 in the presence of PB1 and PA, or was able to partially disrupt the complexes. The sera were accordingly likely to serve as useful inhibitory probes of the enzymatic functions of the influenza virus polymerase.

**Inhibition of the virion transcriptase**

Preliminary experiments indicated that crude serum non-specifically inhibited in vitro transcription by the virion polymerase. We therefore purified immunoglobulin G (IgG) fractions from the sera by chromatography on
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DEAE Affi-Gel Blue Sepharose (Hanecak et al., 1982). We then tested the ability of the purified sera to inhibit the virion-associated polymerase, using globin mRNA as a model primer. As shown in Fig. 3 (a), serum F5 decreased polymerase activity by 90% when added at a concentration of 0.4 mg/ml, whereas sera F1–F4 did not substantially inhibit transcription. Polyacrylamide gel analyses of the products synthesized in the presence of F5 showed that the residual enzyme activity still produced full-length transcripts of the eight vRNA segments (data not shown), indicating that the inhibitory effects of the serum could not be ascribed simply to RNAse contamination. In addition, the inhibition was likely to be specific because a variety of other sera, including a preimmune sample from the same rabbit did not affect transcription (Fig. 3; pF5), while sera from a duplicate rabbit immunized with the F5 fusion protein was also inhibitory (not shown). Therefore antisera directed against the C terminus of PB2 is capable of interfering with mRNA synthesis.

Since PB2 is known to be responsible for binding the 5'-cap structures of priming mRNAs, it was of interest to test the ability of the antisera to inhibit transcription primed by the dinucleotide ApG in the presence and absence of free cap structure. As with globin-primed transcription, antisera F1, F2, F3 and F4 had little effect on ApG-primed polymerase activity, whether or not cap analogue was included in the reactions, with the addition of up to 2.8 mg/ml of IgG failing to inhibit transcription by more than 25% (Fig. 3b, c). The F5 IgG still inhibited polymerization primed by ApG, although to a lesser degree than mRNA-primed transcription, decreasing enzyme activity by less than 50% (cf 90%) when added at a concentration of 0.4 mg/ml (Fig. 3b). Moreover, further addition of IgG did not result in substantially more inhibition, suggesting that antibody levels were not limiting and that ApG-primed transcription was indeed less sensitive to the effects of the antibody. Similarly, when transcription was primed by ApG in the presence of cap analogue, the maximum decrease in transcription in the presence of the F5 serum was less than 50%, with the degree of inhibition apparently saturating at relatively low antibody concentrations (Fig. 3c). Therefore dinucleotide-primed transcription was less sensitive to the effects of F5 irrespective of the presence of free mRNA 5'-cap structure.
The F5 serum inhibits a step prior to transcription initiation

The fact that the potency of the F5 sera depended on priming conditions could perhaps be explained by the antisera inhibiting an early step in polymerase activity. We therefore tested the ability of the sera to block events prior to the initiation of transcription, using a cap-dependent endonuclease assay (Hagen et al., 1994). These experiments (Fig. 4) examine the ability of the polymerase to bind to and cleave a synthetic mRNA like substrate that is uniquely labelled in the 5' cap structure. As previously shown (Hagen et al., 1994), in the absence of antibody, viral cores catalysed the endonucleolytic cleavage of the capped RNA substrate to release an 11 bp product (Fig. 4a, lane 1). Interestingly, addition of the F5 antisera substantially reduced the amount of cleaved RNA produced by the polymerase cores (Fig. 4a, lane 7), to around 35% of the level seen in the absence of serum (Fig. 4b). This inhibition did not seem to be a non-specific effect of rabbit serum, since the other anti-PB2 sera, as well as similar antisera raised against the PB1 and PA subunits, had only a minimal effect on endonuclease activity (Fig. 4a, lanes 2-6, lane 8; quantification data in Fig. 4b).

To further examine the inhibitory effect of the F5 serum, we purified IgG from the serum and then prepared Fab fragments. As negative controls, similar preparations were made from the anti-PB1 and PA sera. Whole serum, IgG and Fab fragments were then compared for their ability to inhibit endonuclease activity. As before, whole F5 anti-PB2 serum markedly decreased cleavage of the capped RNA substrate, whereas the anti-PB1 and PA sera had little effect (Fig. 5a, lanes 1-4). A similar pattern of inhibition was obtained with purified IgG (lanes 5-7) and with Fab fragments (lanes 8-10), with only F5 anti-PB2-derived material causing a substantial decrease in endonuclease activity. The inhibitory activity of the F5 IgG was apparently not affected by cleavage into Fab fragments, as all three forms of the serum caused a similar decrease in RNA cleavage (to around 35% of that seen in the absence of serum). We concluded therefore that the F5 serum was capable of substantially inhibiting the influenza virus transcriptase prior to transcription initiation. In addition, although the inhibition was mediated by IgG, it did not require divalent antibody.

The F5 serum does not inhibit 5' cap structure recognition by PB2

Although the F5 serum reduced cleavage of a capped RNA substrate by the polymerase, the inhibition could result from interference with the endonucleolytic cleavage itself, or from interference with RNA substrate binding. The main determinant of substrate recognition is thought to be the 5' mRNA cap structure (Bouloy et al., 1980) and interestingly, the region of PB2 contained in the F5 fusion protein overlaps with an area proposed to share sequence similarity with cellular cap-binding proteins (Fig. 1; de la Luna et al., 1989). Therefore it was possible that the F5 serum was interfering with cap structure recognition by the polymerase. Accordingly, we tested the effect of the sera on cap binding by PB2, using the assay developed by Blaas et al. (1982a,b), in which PB2 can be specifically radiolabelled with a photoactivatable cap analogue. When detergent-disrupted virus was incubated with the cap analogue, and the samples irradiated with UV light, the PB2 protein became radiolabelled in a reaction that could be blocked by an excess of unlabelled competitor cap analogue (Fig. 6, lanes 1 and 2). Labelling of the nucleoprotein that also occurred under these conditions is thought to be a non-specific consequence of the abundance of the protein (Blaas et al., 1982a, b). However, none of the antisera, including F5, reduced the intensity of labelling of PB2 (Fig. 6, lanes 3-7), indicating that they did not interfere with cap-binding. This is also consistent with the observation that the F5 serum did not differentially inhibit ApG and ApG + cap structure primed synthesis (Fig. 3). Therefore it seems likely that the F5 serum inhibits the endonuclease activity of the influenza polymerase.
Discussion

Several distinct steps can be identified in the process of influenza virus mRNA synthesis that could potentially be affected by immunoglobulins. The initial step occurs with the recognition of the 5' cap structure of the priming mRNA by the PB2 protein (Ulmanen et al., 1981; Blaas et al., 1982a, b; Penn et al., 1982; Cianci et al., 1995), which probably results in a conformational change in the polymerase, as free cap analogue is an allosteric activator of ApG-primed synthesis (Stridh et al., 1981; Penn & Mahy, 1984; Kawakami et al., 1985). Next, the primer is endonucleolytically cleaved by the polymerase (Plotch et al., 1981) and transcription is initiated by the addition of the first nucleotide in a process catalysed by PB1 (Ulmanen et al., 1981; Braam et al., 1983; Romanos & Hay, 1984). Sometime after the first 11–15 nucleotides have been added, PB2 releases the cap structure (Braam et al., 1983) and elongation proceeds until the polyadenylation site is reached, where a poly(A) tail is believed to be added by a process of polymerase stuttering (Krug et al., 1989; Luo et al., 1991; Li & Palese, 1994). Our data indicate that the F5 serum preferentially inhibits endonucleolytic cleavage of the capped mRNA primer. Cleavage of a capped substrate was markedly reduced in the presence of F5 serum (Figs 4 and 5), which could result from direct inhibition of the nuclease, or indirectly from blocking of substrate recognition. However, as the serum showed no ability to interfere with cap recognition as assayed by transcription stimulation or direct binding (Figs 3 and 6), and the 5' cap structure is thought to be a major determinant of substrate recognition (Bouloy et al., 1980), we conclude that the serum most likely inhibits endonuclease activity. It is possible the serum inhibits later steps in transcription as well, since a greater reduction was seen in overall mRNA-primed synthesis than in endonuclease activity (90% compared with 65%; Figs 3 and 4; although the two assays may not be directly comparable). The fact that F5 also inhibited ApG-primed transcription could indicate an effect on transcription initiation or elongation, as it might be expected that under these priming conditions endonuclease activity is non-essential. However, it is also possible that events necessary for mRNA-primed transcription (such as endonuclease activity or associated conformational changes in the polymerase) are not completely decoupled from transcription during ApG-primed synthesis. Consistent with the latter possibility, several ts virus mutants have been found with lesions in the PB2 gene that affect dinucleotide-primed transcription (Kendal et al., 1979; Nichol et al., 1981). Nevertheless, the primary effect of the F5 serum on normally primed synthesis seems to be inhibition of the endonuclease.

Interestingly, two recently published studies have also examined the effect of anti-PB2 immunoglobulins on polymerase activity. One study identified a monoclonal antibody (MAb) directed against PB2 that affected mRNA-primed transcription initiation, but with a different mode of action to the F5 serum (Barcena et al., 1994). Based on a study of the relative efficiencies of transcription initiation seen with two mRNA primers and either CTP or GTP as the initiating nucleotide, the authors concluded that the MAb did not inhibit an enzymatic activity of the polymerase but instead modified the process of primer selection. The most recent study examined the effect of polyclonal sera raised against the entire sequence of PB2, and found that similarly to F5, the serum blocked endonucleolytic cleavage of a capped RNA substrate (Shi et al., 1995). While our results are consistent with this observation, Shi and colleagues did not examine whether their antiserum directly inhibited...
endonuclease activity, or acted indirectly by preventing substrate recognition. Therefore the possibility remains that the two sera act by different methods.

Several possibilities exist as to the mechanism by which the F5 serum inhibits endonuclease activity. The transcriptase inhibitory activity must arise from immunoglobulins reactive against amino acids 583–759 of PB2, as this polypeptide region was used as the original immunogen (Fig. 1) in two rabbits that both produced inhibitory sera (data not shown). Other evidence that this inhibition is specific to antibodies reactive with the carboxy terminus of PB2 comes from the fact that F5 is the only one out of the five different PB2-specific sera to exhibit this activity. Although polyclonal sera to three other regions of PB2 recognized the polymerase complex based on their ability to efficiently immunoprecipitate all three P proteins, they exhibited little effect on transcription. Therefore the carboxy terminus of PB2 may be directly involved in endonuclease activity, and inhibition results from the binding of IgG to part of the protein directly involved in catalysis. Indeed, Shi and colleagues concluded that PB2 was the endonuclease on the basis of antibody-mediated inhibition of substrate cleavage (Shi et al., 1995). As discussed above, our findings are not only consistent with, but also strengthen such a conclusion, as we have shown that the decrease in endonuclease activity caused by the F5 serum probably did not arise as an indirect consequence of blocking caps-structure recognition. However, although the most direct interpretation of our data is that PB2 is responsible for endonucleolytic cleavage of capped RNAs, the possibility that the inhibition results from steric interference with another component (or components) of the polymerase more directly responsible for endonuclease function can not be discounted. Although Fab fragments of F5 IgG retained inhibitory activity (Fig. 5), a Fab moiety is still a relatively bulky molecule that could conceivably act via a steric mechanism. Definitive identification of PB2 as the endonuclease will require further experimentation.

The relative inability of the F1 serum to precipitate polymerase complexes suggested that either the anti-serum disrupted the interactions between PB2 and the other P proteins, or that the epitopes recognized by the sera (within amino acids 1–152) were less accessible in the presence of PB1 and PA. The latter explanation seems more likely, as numerous studies have noted the necessity of all three P proteins for enzymatic activity (Szewczyk et al., 1988; Huang et al., 1990; Kobayashi et al., 1992; Hagen et al., 1994; Shi et al., 1995). Therefore if the serum disrupted polymerase complexes, it would be expected to inhibit polymerase function, which was not the case. However, not all of the N-terminal residues of PB2 can be occluded in polymerase complexes, as an epitope recognized by a MAb that affects primer selection has been mapped to amino acids 1–113 (Barcena et al., 1994; Ochoa et al., 1995).

Although the F5 serum was evidently the most effective in immunoprecipitation reactions (Fig. 2), we feel that the failure of sera F2, F3, and F4 to inhibit the polymerase is not necessarily due to an insufficient antibody titre, but perhaps reflects the crucial role of the region recognized by the F5 serum. In support of this hypothesis, we have raised similar sets of region-specific polyclonal sera against the PB1 and PA proteins, several of which are as effective in immunoprecipitation reactions as the anti-PB2 F5 serum (Digard et al., 1989, and data not shown). None of the 10 anti-PB1 and PA sera so produced were able to inhibit the virion transcriptase (data not shown). This was particularly surprising for the anti-PB1 sera, given the central role of this protein in transcription. We also note that another study found that of 13 anti-PA MAbs and 8 anti-PB2 MAbs only one antibody directed against PB2 was able to affect polymerase activity (Barcena et al., 1994). Therefore it seems that the virion transcriptase is relatively resistant to antibody-mediated inhibition, which in turn suggests that the C terminus of PB2 may prove a fruitful target for further structural and genetic analysis.

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