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An influenza virus gene encoding two different proteins

By S. C. Inglis† and J. W. Almond‡
† *Division of Virology, Department of Pathology, University of Cambridge, Laboratories Block, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K.*
‡ *Sandoz Forschungsinstitut G.m.b.H., Brunnerstrasse 59, A-1235 Wien, Postfach 80, Austria*

Evidence is presented which confirms that the influenza virus genome specifies a polypeptide of molecular mass 11000, in addition to the eight previously recognized gene products. A summary is included of results that show that this polypeptide is encoded by the smallest genome segment of the virus (segment 8) which also encodes a polypeptide of molecular mass 23000 (NS_1). The implications of these findings are considered.

Introduction

It is now well established that the genome of influenza virus consists of eight separate RNA molecules (McGeoch *et al.* 1976; Palese & Schulman 1976; Pons 1976). Various techniques have been used to show that each RNA codes for a separate virus polypeptide (reviewed by Barry & Mahy 1979); the eight recognized virus gene products range from 23000 to 95000 in molecular mass and include seven structural (P1, P2, P3, M, NP, NA, HA) and one non-structural (NS_1) polypeptides (Inglis *et al.* 1976). The obvious implication that each genome segment represents a single gene was therefore difficult to reconcile with the possibility, recognized a number of years ago, that the virus encodes an additional polypeptide of molecular mass 11000 (Skehel 1972).

Most workers agree that the synthesis of such a polypeptide is induced in influenza virus infected cells (Skehel 1972; Follet *et al.* 1974; Minor & Dimmock 1975; Etkind & Krug 1975; Lamb *et al.* 1978; Bosch *et al.* 1978), but in quantities that vary depending on the virus and cell type used. The polypeptide is not a major component of purified virions (Skehel & Schild 1971; Klenk *et al.* 1972), and so has been referred to as a possible second non-structural polypeptide (NS_2). Recently, Lamb *et al.* (1978) showed that the polypeptide was present in purified virus of the WSN strain of human influenza, but since the amount was very small, it is unlikely that the polypeptide contributes significantly to the structure of the virion, and therefore it seems reasonable to retain the name NS_2 at least for the present.

Two lines of evidence suggest that NS_2 does not arise through proteolytic cleavage of another virus polypeptide. First, the tryptic peptide map of NS_2 is unrelated to those of the other virus polypeptides (Lamb *et al.* 1978, and this symposium; Inglis 1978) and second, a polyadenylated mRNA that directs the synthesis of NS_2 *in vitro* can be separated from other virus specific mRNAs by gradient sedimentation (Stephenson *et al.* 1977; Inglis 1978). These observations led to the suggestion (Lamb *et al.* 1978) that NS_2 is a separate virus gene product. However, the possibility that NS_2 is a cell-coded polypeptide whose synthesis is induced by virus infection had not been excluded completely.

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In this report, we summarize results that show that the NS_2 polypeptide is indeed virus-specific and that it is encoded by the same genome segment that specifies the NS_1 polypeptide (segment 8). The implications of these findings are discussed.

NS_2 IS A VIRUS-CODED POLYPEPTIDE

Figure 1*a* shows the polypeptides synthesized in chick embryo fibroblasts (CEF) that have been infected with either the Rostock or Dobson strain of the avian influenza, fowl plague virus (FPV), or with the WSN strain of human influenza virus. Each virus strain induced the synthesis of a polypeptide of molecular mass 11000 (labelled NS_2). If this polypeptide were cell-coded, then it should be identical regardless of which virus strain were used to initiate infection.

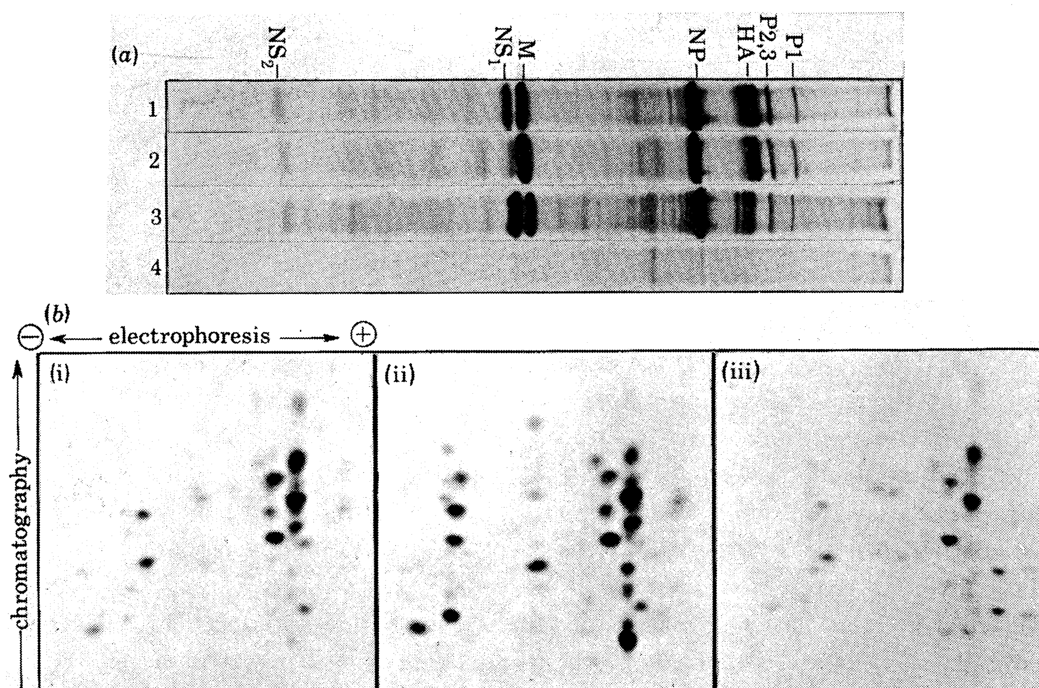

FIGURE 1. (*a*) Polyacrylamide gel electrophoresis (from right to left) of polypeptides synthesized in influenza virus-infected CEF. Track 1, infected with FPV-Rostock; track 2, infected with FPV-Dobson; track 3, infected with human influenza-WSN; track 4, mock-infected. Cells were labelled for 30 min with [^{35}S]methionine at 4.5 h after infection and analysed on a polyacrylamide gel (165 g/l). Labelled polypeptides were detected by autoradiography. Conditions for infection, labelling, and gel electrophoresis were as described previously (Inglis *et al.* 1976). (*b*) Two-dimensional tryptic peptide maps of the NS_2 polypeptides induced in CEF by infection with (i) FPV-Rostock, (ii) FPV-Dobson and (iii) human influenza WSN. The procedure for peptide mapping was exactly as described previously (Inglis *et al.* 1976). [^{35}S]methionine-labelled peptides were separated by electrophoresis at pH 6.5 followed by ascending chromatography, and were detected by autoradiography.

On the other hand, a virus-specific polypeptide might be expected to show strain-specific differences. Therefore we prepared two-dimensional tryptic peptide maps of the NS_2 polypeptides shown in figure 1*a*; these are shown in figure 1*b*. The three peptide maps are broadly similar, but show several differences. The differences were found to be reproducible in separate experiments, and were confirmed by analysis of mixed samples (data not shown). These results therefore argue strongly that NS_2 is a virus-coded polypeptide.

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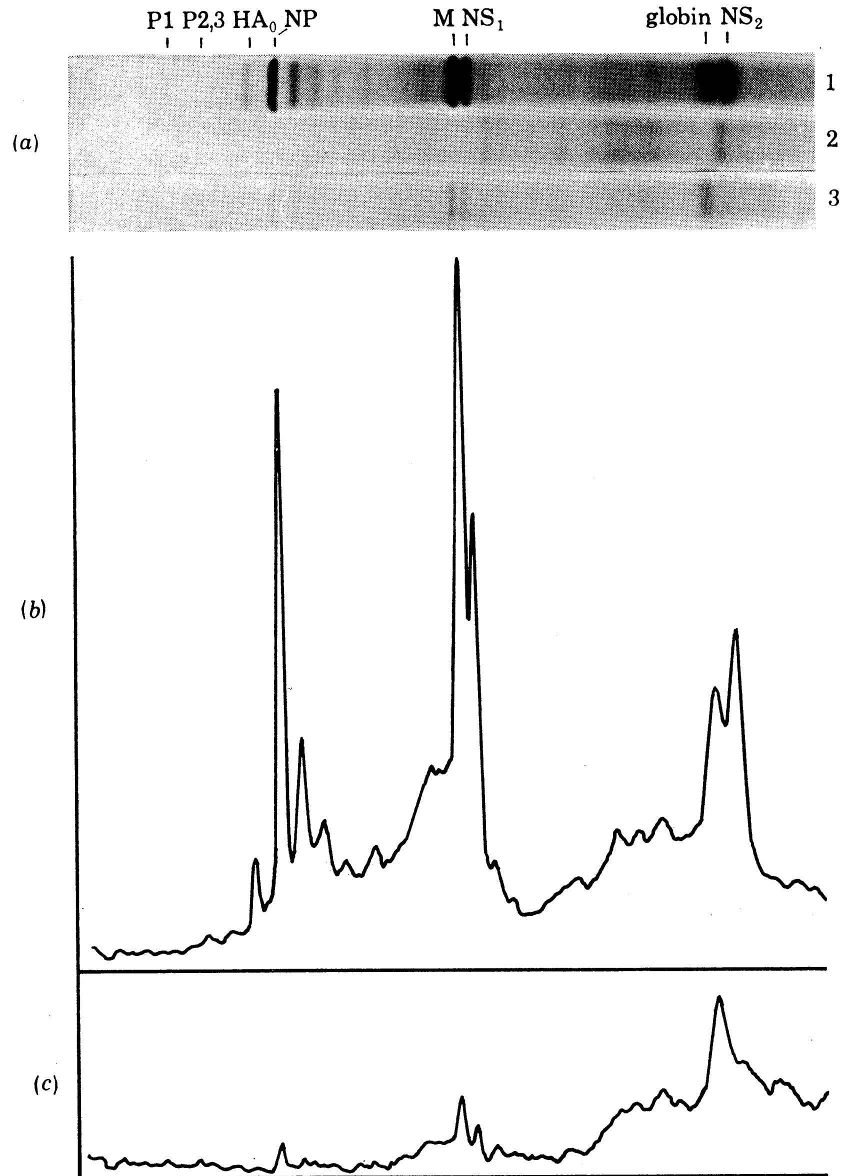

FIGURE 2. (*a*) Polyacrylamide gel electrophoresis of polypeptides synthesized *in vitro* by a cell-free wheat germ system: 1 μg of poly(A)-containing RNA from FPV-infected CEF was mixed with 0.05 μg of globin mRNA and annealed in the presence or absence of 5 μg of RNA extracted from purified FPV (vRNA). Annealed mixtures were ethanol-precipitated, dissolved in 10 μl of H_2O and added to the wheat germ system at a 1 in 10 dilution. Polypeptides synthesized by the system in response to added RNA were labelled with [^{35}S]methionine, separated on a polyacrylamide gel (175 g/l), and detected by autoradiography. Track 1, translation products of mRNA annealed in the absence of vRNA; track 2, endogenous reaction products; track 3, translation products of mRNA that had been annealed in the presence of vRNA. Procedures for preparation of mRNA, annealing of mRNA and cell-free translation were as detailed previously (Inglis *et al.* 1977). Characterization of the virus-specific cell-free translation products has been described elsewhere (Inglis *et al.* 1977). (*b*) Densitometer scan of the autoradiogram shown in (*a*), track 1. (*c*) Densitometer scan of the autoradiogram shown in (*a*), track 3.

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Hybridization of virus-specific mRNA with RNA extracted from purified virions provided further evidence in support of this conclusion. Poly(A)-containing RNA from FPV-infected CEF was annealed in the presence and absence of vRNA, precipitated with ethanol and added directly to a cell-free protein synthesizing system derived from wheat germ. Under these conditions, mRNAs complementary in sequence to genome RNA are converted into a double-stranded form, and so are no longer active in the cell-free system (Inglis *et al.* 1977). A small amount of globin mRNA was included in the annealing reaction to act as an internal control. The cell-free translation products of the annealed RNA mixtures are shown in figure 2. The presence of vRNA in the annealing mixture resulted in a specific reduction in the synthesis of NS_2, as well as the other virus polypeptides, relative to globin (compare tracks 1 and 3 in (*a*), and corresponding densitometer scans in (*b*) and (*c*) respectively). This experiment shows directly that the mRNA for NS_2 is complementary to sequences present in the virus genome. These results indicate that NS_2 is certainly a virus-specific polypeptide, and, taken together with the observation that NS_2 is apparently unrelated to the other virus polypeptides (Inglis 1978; Lamb *et al.* 1978), and that it is encoded by a separate species of mRNA (Stephenson *et al.* 1977; Inglis 1978), they suggest that NS_2 is the product of a ninth virus gene.

The NS_2 and NS_1 polypeptides are encoded by the same genome segment

The strain of FPV used in these experiments contains only eight genome RNA segments (McGeoch *et al.* 1976), and protein coding functions have already been ascribed to each of these (Inglis *et al.* 1977; Almond *et al.* 1977, 1979). We therefore concluded that NS_2 is encoded by a genome segment that also specifies another polypeptide. Three kinds of experiment indicated that the smallest genome segment (segment 8), which is known to code for the NS_1 polypeptide, fulfils this role.

(1) Messenger RNA extracted from FPV-infected cells was annealed in the presence and absence of purified segment 8 RNA, and subsequently translated *in vitro*. Under these conditions, synthesis of both the NS_1 and NS_2 polypeptides was specifically inhibited (Inglis *et al.* 1979).

(2) The mRNAs encoding the NS_2 and NS_1 polypeptides were separated by density gradient sedimentation. The distribution across the gradient of sequences complementary to segment 8 was then analysed by hybridization with ^{125}I-labelled segment 8 RNA. Complementary sequences were most abundant in two parts of the gradient, one of which contained the NS_1 mRNA, and the other the NS_2 mRNA (Inglis *et al.* 1979).

(3) Tryptic peptide maps were prepared from the NS_2 polypeptides specified by a series of viruses produced by recombination between the Rostock and Dobson strains of FPV. The parental origin of the genome segments in each of these recombinants had been established as described previously (Almond *et al.* 1977; Almond & Barry 1979). Since the peptide maps of the Rostock and Dobson specified NS_2 polypeptides are different, it was possible to determine for each recombinant the parental origin of its NS_2. The results of this experiment will be presented in full elsewhere (Inglis *et al.* 1979), but are summarized in table 1. The presence of the Rostock or Dobson specific NS_2 polypeptide correlated perfectly with the presence of the corresponding Rostock or Dobson specific genome segment 8. This correlation was not apparent for any other genome segment. These data provided confirmation that the genetic information for the NS_2 polypeptide resides in genome segment 8.

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Our experiments indicate that two different sized mRNAs, which separately code for the NS_1 and NS_2 polypeptides, are produced in the infected cell by transcription of genome segment 8. However, the NS_1 mRNA appears to represent the entire length, except for a short stretch of about 30 nucleotides at the 5′ end, of segment 8 (Skehel & Hay 1978). Therefore, if, as the peptide maps suggest, the NS_1 and NS_2 polypeptides are completely unrelated, almost all of the nucleotide sequences in the NS_2 mRNA must also be present in the NS_1 mRNA, but remain untranslated. This could occur in two ways. First, the NS_2 cistron might be contiguous

Table 1.

virus used	parental origin of genome segment encoding polypeptide†								parental origin of NS_2
	P1	P2	P3	HA	NP	NA	M	NS_1	
parent									
Rostock	R	R	R	R	R	R	R	R	R
recombinants									
Di47-2	R	R	R	R	R	R	R	D	D
Di34-3	R	R	R	R	D	R	R	R	R
Di44-2	R	D	R	R	D	R	R	D	D
Di44-4	R	D	R	D	D	R	D	R	R
Di44-7	R	D	R	R	D	D	D	D	D
DimN5c-4	D	D	R	D	D	R	?	D	D
Dd45-g	D	R	D	D	D	?	?	R	R
parent									
Dobson	D	D	D	D	D	D	D	D	D

† P polypeptide nomenclature is based on the order of gel migration of the polypeptides in the Rostock strain (P1 is largest, etc.). It should be noted that P2 and P3 of Rostock are functionally equivalent to P3 and P2 respectively of Dobson (Almond & Barry 1979). Therefore, where Dobson P3 replaces Rostock P2 in a recombinant, D is included under the column headed 'P2'. Likewise a D is shown in the column headed 'P3', when Rostock P3 is replaced by Dobson P2.

with that of NS_1, but fail to translate through having a 'closed' initiation site for protein synthesis. Several virus mRNAs are known to contain untranslated cistrons (reviewed by Revel & Groner 1978). Alternatively, the NS_2 cistron could be contained partly or wholly within the NS_1 coding sequence, but in a different codon-reading frame. Again the initiation site for NS_2 polypeptide synthesis would be unrecognized in the NS_1 mRNA. There are also precedents for this kind of structure in other viruses. Nucleotide sequences which code in two and even three different reading frames have been discovered in the DNA of bacteriophages ϕX174 and G4 (Barrell *et al.* 1976; Shaw *et al.* 1978) and of the animal virus SV40 (Contreras *et al.* 1977).

Considering the sizes of the molecules involved, the second of these possibilities seems more likely. Genome segment 8 contains about 820 nucleotides (McGeoch *et al.* 1976), which could encode a protein of a maximum molecular mass of about 30000. The maximum is likely to be somewhat smaller than this, since about 30 nucleotides from the 5′-terminus of segment 8 are not represented as mRNA (Skehel & Hay 1978), and since the first potential initiation sequence for translation (the anticodon 3′-UAC-5′) occurs 27 nucleotides from the 3′ end of segment 8 (Skehel & Hay 1978; Robertson, this symposium). If the estimated sizes of the NS_1 and NS_2 polypeptides (which give a combined molecular mass of 34000) are accurate then it is probable that at least part of the NS_2 coding sequence lies within the NS_1 cistron.

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The mechanism by which the NS_2 mRNA is synthesized in the infected cell is unknown, but it is clear that its production is controlled separately from that of NS_1 mRNA. During primary transcription of the infecting virus genome by the virion polymerase (either *in vivo* in the presence of cycloheximide, or *in vitro*) NS_2 mRNA, unlike NS_1 mRNA and all the other virus mRNAs, is not produced (Stephenson *et al.* 1977; Lamb *et al.* 1978). Synthesis of the NS_2 polypeptide begins later in the infectious cycle than that of NS_1 (Inglis 1978). This seems to reflect differential synthesis of the two types of mRNA at early and late times after infection (Inglis, unpublished observations). Independent control of production of NS_1 and NS_2 mRNAs could be achieved in two fundamentally different ways. The NS_2 and NS_1 mRNAs could be transcribed separately from independent promoter sites on genome segment 8. Recognition of the NS_2 promoter site might then be dependent on the production of a new or modified transcriptase enzyme late in infection. Two separate sites for the initiation of transcription are thought to exist on the 42*S* minus strand RNA of the alphaviruses (Brzeski & Kennedy 1978). On the other hand, only the full length NS_1 mRNAs might be transcribed directly from segment 8. These transcripts could be processed, perhaps by enzyme activities produced late in infection, to form functional NS_2 mRNAs. Examples of this kind of mRNA synthetic mechanism also have been demonstrated among the animal viruses (Chow *et al.* 1977; Bratosin *et al.* 1978).

The biological role of the NS_2 polypeptide is completely unknown. However, another virus polypeptide can scarcely be considered excessive to the demands of a virus whose replication cycle involves so many apparently virus-specified enzyme activities (e.g. RNA polymerases to synthesize early mRNA, late mRNA, template cRNA and vRNA, as well as cap transferase and poly(A) polymerase activities). Clearly, the functional significance of the NS_2 polypeptide requires further investigation.

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References (Inglis & Almond)

Almond, J. W. & Barry, R. D. 1979 Genetic recombination between two strains of fowl plague virus: construction of genetic maps. *Virology* **92**, 407–415.

Almond, J. W., McGeoch, D. & Barry, R. D. 1977 Method for assigning temperature sensitive mutations of influenza viruses to individual segments of the genome. *Virology* **81**, 62–73.

Almond, J. W., McGeoch, D. & Barry, R. D. 1979 Temperature-sensitive mutants of fowl plague virus: isolation and genetic characterisation. *Virology* **92**, 416–427.

Barrell, B. G., Air, G. M. & Hutchinson III, C. A. 1976 Overlapping genes in bacteriophage ϕX174. *Nature, Lond.* **264**, 34–41.

Barry, R. D. & Mahy, B. W. J. 1979 The influenza genome and its replication. *Br. med. Bull.* **35**, 39–46.

Bosch, F. X., Hay, A. J. & Skehel, J. J. 1978 RNA and protein synthesis in a permissive and an abortive influenza virus infection. In *Negative strand viruses and the host cell* (ed. B. W. J. Mahy & R. D. Barry), pp. 465–474. London: Academic Press.

Bratosin, S., Horowitz, M., Laub, O. & Aloni, Y. 1978 Electron microscopic evidence for splicing of SV40 late mRNAs. *Cell* **13**, 783–790.

Brzeski, H. & Kennedy, S. I. T. 1978 Synthesis of alphavirus-specified RNA. *J. Virol.* **25**, 630–640.

Chow, L. T., Gelinas, R. E., Broker, T. R. & Roberts, R. J. 1977 An amazing sequence arrangement at the 5′ ends of adenovirus 2 messenger RNA. *Cell* **12**, 1–8.

Contreras, R., Rogiers, R., Van de Voorde, A. & Fiers, W. 1977 Overlapping of the VP2–VP3 gene and the VP1 gene in the SV40 genome. *Cell* **12**, 529–538.

Etkind, P. R. & Krug, R. M. 1975 Purification of influenza viral complementary RNA. Its genetic content, and activity in wheat germ cell-free extracts. *J. Virol.* **16**, 1464–1475.

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Follet, E. A. C., Pringle, C. R., Wunner, W. H. & Skehel, J. J. 1974 Virus replication in enucleate cells: Vesicular stomatitis virus and influenza virus. *J. Virol.* **13**, 394–399.
Inglis, S. C. 1978 The synthesis of influenza virus proteins. Ph.D. thesis, University of Cambridge.
Inglis, S. C., Barrett, T., Brown, C. M. & Almond, J. W. 1979 The smallest genome RNA segment of influenza virus contains two genes, which may overlap. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3790–3794.
Inglis, S. C., Carroll, A. R., Lamb, R. A. & Mahy, B. W. J. 1976 Polypeptides specified by the influenza virus genome. 1. Evidence for eight distinct gene products. *Virology* **74**, 489–503.
Inglis, S. C., McGeoch, D. & Mahy, B. W. J. 1977 Polypeptides specified by the influenza virus genome. 2. Assignment of protein coding functions to individual genome segments by *in vitro* translation. *Virology* **78**, 522–536.
Klenk, H.-D., Rott, R. & Becht, H. 1972 On the structure of the influenza virus envelope. *Virology* **47**, 579–597.
Lamb, R. A., Etkind, P. R. & Choppin, P. W. 1978 Evidence for a ninth influenza viral polypeptide. *Virology* **91**, 60–78.
McGeoch, D., Fellner, D. & Newton, C. 1976 Influenza virus genome consists of eight distinct RNA species. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3045–3049.
Minor, P. & Dimmock, N. J. 1975 Inhibition of synthesis of influenza virus proteins. Evidence for two host cell-dependent events during multiplication. *Virology* **67**, 114–123.
Palese, P. & Schulman, J. L. 1976 Differences in RNA patterns of influenza A viruses. *J. Virol.* **17**, 876–884.
Pons, M. W. 1976 A re-examination of influenza single and double-stranded RNAs by gel electrophoresis. *Virology* **69**, 789–792.
Revel, M. & Groner, Y. 1978 Post transcriptional and translational controls of gene expression in eukaryotes. *A. Rev. Biochem.* **47**, 1079–1126.
Shaw, D. C., Walker, J. E., Northrop, F. D., Barrell, B. G., Godson, G. N. & Fiddes, J. C. 1978 Gene K, a new overlapping gene in bacteriophage G4. *Nature, Lond.* **272**, 510–515.
Skehel, J. J. 1972 Polypeptide synthesis in influenza virus-infected cells. *Virology* **49**, 23–36.
Skehel, J. J. & Hay, A. J. 1978 Nucleotide sequences at the 5′ termini of influenza virus RNAs and their transcripts. *Nucl. Acids Res.* **4**, 1207–1218.
Skehel, J. J. & Schild, G. C. 1971 The polypeptide composition of influenza A viruses. *Virology* **44**, 396–408.
Stephenson, J. R., Hay, A. J. & Skehel, J. J. 1977 Characterisation of virus-specific messenger RNAs from avian fibroblasts infected with fowl plague virus. *J. gen. Virol.* **36**, 237–248.

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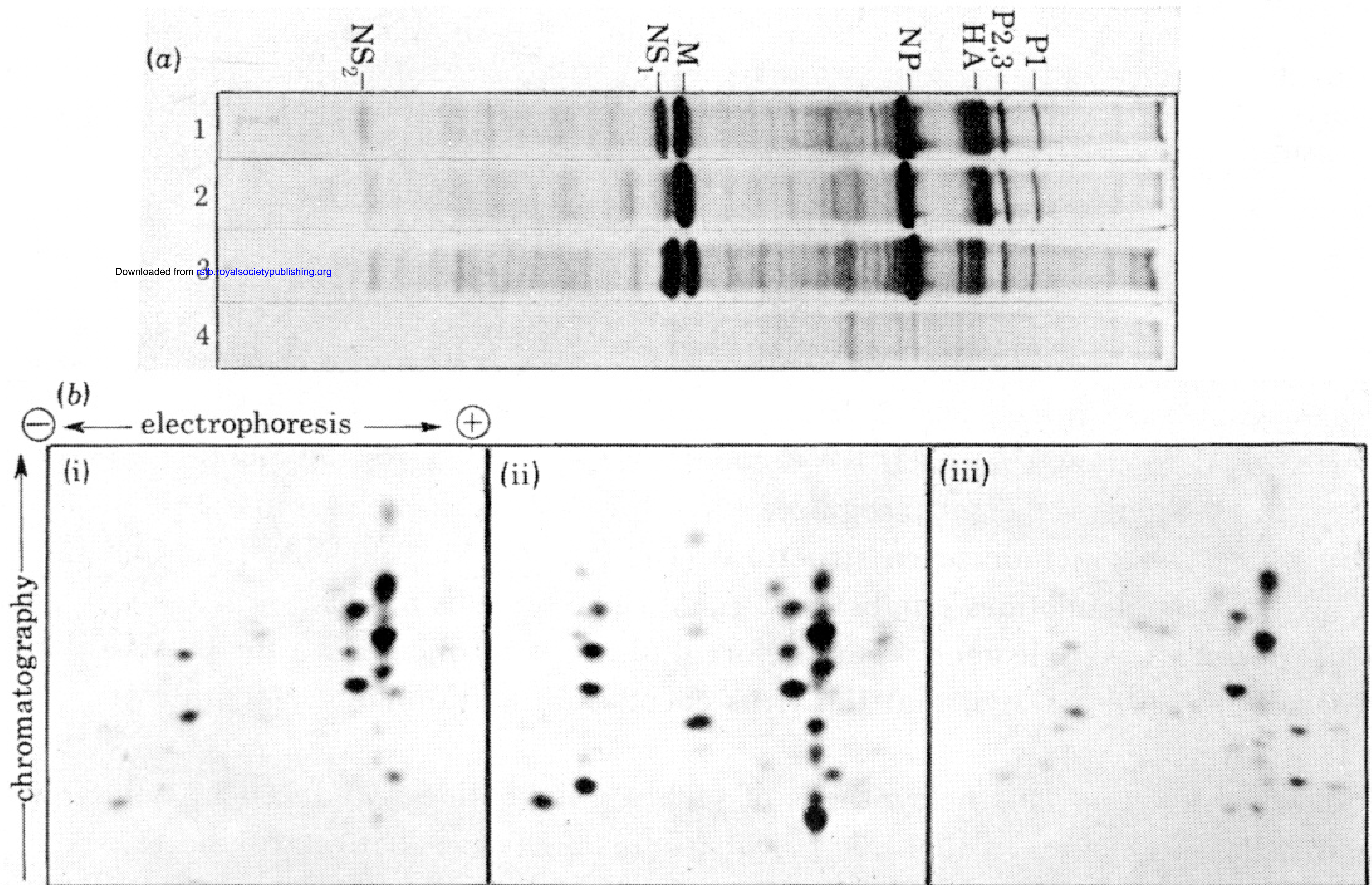

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FIGURE 1. (*a*) Polyacrylamide gel electrophoresis (from right to left) of polypeptides synthesized in influenza virus-infected CEF. Track 1, infected with FPV-Rostock; track 2, infected with FPV-Dobson; track 3, infected with human influenza-WSN; track 4, mock-infected. Cells were labelled for 30 min with [^{35}S]methionine at 4.5 h after infection and analysed on a polyacrylamide gel (165 g/l). Labelled polypeptides were detected by autoradiography. Conditions for infection, labelling, and gel electrophoresis were as described previously (Inglis *et al.* 1976). (*b*) Two-dimensional tryptic peptide maps of the NS$_2$ polypeptides induced in CEF by infection with (i) FPV-Rostock, (ii) FPV-Dobson and (iii) human influenza WSN. The procedure for peptide mapping was exactly as described previously (Inglis *et al.* 1976). [^{35}S]methionine-labelled peptides were separated by electrophoresis at pH 6.5 followed by ascending chromatography, and were detected by autoradiography.

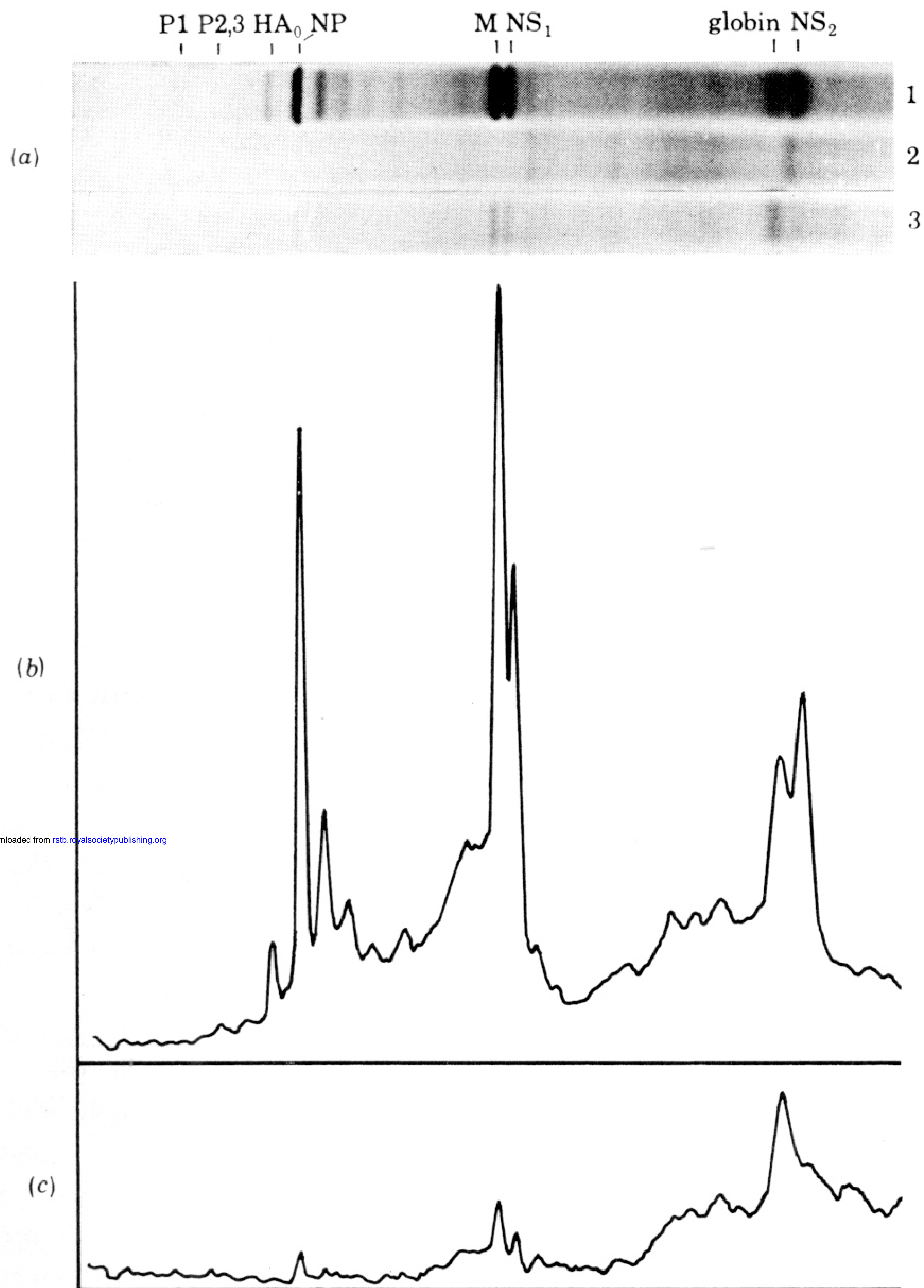

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FIGURE 2. (*a*) Polyacrylamide gel electrophoresis of polypeptides synthesized *in vitro* by a cell-free wheat germ system: 1 μg of poly(A)-containing RNA from FPV-infected CEF was mixed with 0.05 μg of globin mRNA and annealed in the presence or absence of 5 μg of RNA extracted from purified FPV (vRNA). Annealed mixtures were ethanol-precipitated, dissolved in 10 μl of H_2O and added to the wheat germ system at a 1 in 10 dilution. Polypeptides synthesized by the system in response to added RNA were labelled with [^{35}S]methionine, separated on a polyacrylamide gel (175 g/l), and detected by autoradiography. Track 1, translation products of mRNA annealed in the absence of vRNA; track 2, endogenous reaction products; track 3, translation products of mRNA that had been annealed in the presence of vRNA. Procedures for preparation of mRNA, annealing of mRNA and cell-free translation were as detailed previously (Inglis *et al.* 1977). Characterization of the virus-specific cell-free translation products has been described elsewhere (Inglis *et al.* 1977). (*b*) Densitometer scan of the autoradiogram shown in (*a*), track 1. (*c*) Densitometer scan of the autoradiogram shown in (*a*), track 3.

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