Non-structural Proteins in Newcastle Disease Virus-infected Cells

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SUMMARY
Examination of pulse-labelled Newcastle disease virus (NDV)-infected chick embryo fibroblasts (CEF) by two-dimensional polyacrylamide gel electrophoresis revealed the presence of two virus-coded non-structural polypeptides of mol. wt. 36K and 33K. Longer pulses and pulse-chase incubations revealed the production of an additional, glycosylated, non-structural polypeptide of mol. wt. 40K (gp40). Kinetic arguments suggest that 36K and 33K are primary translation products but that gp40 is not. 36K was stable in chase incubations, but 33K was not. Partial digest peptide analysis showed that gp40 and an additional glycosylated polypeptide gp62, which is sometimes present (Chambers & Samson, 1980), are related to the HN polypeptide. Partial digest peptide analysis of the 36K polypeptide generated only a few peptides, which were not sufficient to conclude whether 36K was related to the major virus polypeptides, and since polypeptide 33K was metabolically unstable, insufficient radioactivity was incorporated for peptide studies. Extensive strain-dependent variation in the isoelectric points and mol. wt. of all the NDV polypeptides which are soluble in the isoelectric focusing gels, including 36K and 33K, is reported. This variation, and the insensitivity of the synthesis of 36K and 33K to actinomycin D, show that both non-structural polypeptides are virus-coded.

INTRODUCTION
Paramyxoviruses, of which SV5, Sendai and Newcastle disease virus (NDV) are the best-studied examples, contain six virus-coded polypeptides (Kingsbury, 1977) together with some actin derived from the cells in which they were grown. The virus envelope contains three of the virus-coded polypeptides: HN, F and M. Nucleocapsids contain the other three polypeptides: L, NP and P. The mol. wt. of the corresponding polypeptides are similar for Sendai, SV5 and NDV, with the exception of P. The mol. wt. of HN is approx. 75K, F 67K, M 39K, L 200K and NP 55K. The mol. wt. of the P polypeptide is 79K in Sendai virus (Lamb et al., 1976), 53K to 56K in NDV (Chambers & Samson, 1980; Smith & Hightower, 1981 a), and 46K in SV5 (Buetti & Choppin, 1977). The analogy of the 'P' polypeptides is weakened by this variation in mol. wt., and the 'P' polypeptide of NDV was termed NAP (nucleocapsid-associated protein) in an earlier publication (Chambers & Samson, 1980). This terminology is also used in this communication.

The synthesis of all these structural polypeptides is readily detectable in virus-infected cells, and several studies have suggested that non-structural polypeptides are also synthesized. In the case of Sendai virus, it has been shown that the peptide fingerprint of the non-structural polypeptide C (22K) is different from those of the structural polypeptides (Lamb & Choppin, 1978). A polypeptide of similar mol. wt. (24K) has been detected in SV5-infected cells (Peluso et al., 1977). In NDV-infected cells, a non-structural polypeptide, 36K, has been described (Alexander & Reeve, 1972; Hightower & Bratt, 1974; Chambers & Samson, 1980) but not characterized.
Glycosylated non-structural polypeptides are also known. The active F protein is made up of two disulphide-linked polypeptides, F₁ and F₂, derived from a glycosylated precursor F₀ by proteolytic cleavage. HN₀, a precursor to the HN glycoprotein, has been detected for two extremely avirulent NDV strains (Nagai et al., 1976a; Nagai & Klenk, 1977). F₀ and HN₀ may be either structural or non-structural, depending on the virus–cell system (Nagai et al., 1976a). Two glycosylated polypeptides, mol. wt. 62K and 40K (gp62, gp40), have been detected in NDV-infected cells (Chambers & Samson, 1980) but no previous studies have detected similar material in virions.

Experiments were devised to determine whether the NDV-induced non-structural polypeptides are primary gene products or are related to the structural polypeptides.

**METHODS**

*Staphylococcus aureus* V8 protease was obtained from Miles Laboratories, Slough, U.K.; 40% ampholines pH range 5 to 7 were from LKB, South Croydon, U.K.; Nonidet P40 (NP40) was from BDH. Falcon micro-test II plates, 96 flat-bottomed well (96-well plates) were from Becton-Dickinson, Runcorn, U.K. All other materials were as described previously (Chambers & Samson, 1980).

**Virus and cells.** NDV strains AV, HP16, N, LK and Beaudette C, were obtained from Professor C. F. Fox, University of California, Los Angeles. NDV strains B1, F and Texas were obtained from Dr R. Avery, Warwick University. Large scale preparation of purified NDV was as described previously (Chambers & Samson, 1980). Alternatively, for use in labelling experiments, a smaller scale preparation was used. Approximately 10⁵ p.f.u. was injected into each 10-day-old embryonated egg. After 48 h at 37 °C, allantoic fluid was harvested and clarified by centrifugation at 10000 g for 10 min. The supernatant was divided into 1 ml amounts and stored frozen at −70 °C. Virus was used without further purification for labelling experiments on monolayers. Secondary monolayers of chick embryo fibroblasts (CEF) were grown in 96-well plates in medium 199 supplemented with 5% calf serum. BHK cells were grown in medium 199 supplemented with 10% calf serum.

**Radio-labelled virus.** Allantoic membranes from 11-day-old embryonated eggs were collected and transferred to allantoic fluid from NDV-infected eggs (previous section) for 30 min at 37 °C. Membranes were then rinsed three times in Hanks’ salts and transferred to medium 199 containing 0.1 µg/ml leucine. After 6 h incubation at 37 °C, membranes were transferred to medium 199 containing 5 µCi/ml [¹⁴C]leucine and incubated overnight at 37 °C. Medium was pipetted off, mixed with purified non-radioactive NDV, and [¹⁴C]leucine-labelled NDV prepared by centrifugation on sucrose/tartrate gradients (Chambers & Samson, 1980).

[³H]glucosamine-labelled NDV was prepared by a similar procedure, except that media contained 10 µg/ml leucine throughout, and overnight incubation was in medium supplemented with 50 µCi/ml [³H]glucosamine rather than [¹⁴C]leucine.

**Radio-labelled monolayers**

[¹⁴C]leucine-labelled monolayers. Confluent monolayers of CEF or BHK cells in 96-well plates were incubated overnight in medium 199 containing 0.4 µg/ml leucine. Monolayers were rinsed with Hanks’ salts, then incubated at 37 °C for 30 min under 30 µl clarified NDV-infected allantoic fluid, ensuring an m.o.i. greater than 100. After infection, cells were rinsed with Hanks’ salts, then incubated with medium 199 containing 0.1 µg/ml leucine until the labelling period commenced. The monolayers were labelled with 20 µl of medium 199 lacking non-radioactive leucine, supplemented either with 17 µCi/ml [¹⁴C]leucine for labellings of duration greater than 1 h, or with 33 µCi/ml [¹⁴C]leucine for labellings of duration 1 h or less. After labelling, radioactive medium was removed and the monolayer was taken up into
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30 µl of sample buffer [8% (w/v) NP40, 9.5 M-urea, 5% (v/v) 2-mercaptoethanol, 2% (w/v) ampholines pH range 3.5 to 10]. The whole extract was then loaded on to either an isoelectric focusing (IEF) gel or a non-equilibrium pH gradient electrophoresis (NEPHGE) gel (O'Farrell et al., 1977).

[3H]glucosamine-labelled monolayers. The general procedure was as described above, but all media contained 10 µg/ml leucine and labelling was with medium 199 supplemented with 100 µCi/ml [3H]glucosamine. Monolayers were taken up in final sample buffer (Laemmli, 1970).

Actinomycin D, when used, was present in post-infection incubation and labelling media at a final concentration of 2 µg/ml.

Two-dimensional gel electrophoresis. NP40 was used in all IEF procedures, although the virus nucleocapsid protein is less soluble than in gels containing Tween 80 (Chambers & Samson, 1980). The overall results were more reproducible, and the anomalous, slow migration of F in IEF gels containing Tween 80 was avoided (Samson et al., 1981). The general procedure of O'Farrell (1975) was followed, with slight modifications, depending on the isoelectric point (pI) of the polypeptides under study. (i) To resolve basic polypeptides, such as gp40 and 33K, NEPHGE gels contained 2% ampholines pH range 3.5 to 10. These were loaded at the anode (acid) and electrofocused for 2500 V.h. (ii) To resolve neutral and acidic polypeptides, such as HN, F₀, F₁, NAP and 36K, IEF gels contained 1% ampholines pH range 3.5 to 10 and 1% ampholines pH range 5 to 7. These were loaded at the anode and electrofocused for 4000 V.h (Samson et al., 1981). Measurement of pH gradients, equilibration of the IEF gels and running the second dimension were as described by O'Farrell (1975). Second dimensions were run on 10% polyacrylamide gels as described previously (Chambers & Samson, 1980) then either prepared for fluorography (Bonner & Laskey, 1974) or fixed in 10% (v/v) acetic acid and dried down for autoradiography.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE). This was performed on 10% polyacrylamide slab gels (Laemmli, 1970).

Partial digest peptide analysis of NDV polypeptides. NDV-induced polypeptides were located in two-dimensional autoradiographs, cut out, and the gel slices swollen for 15 min in water followed by 30 min in equilibration buffer [0.5% (w/v) SDS, 0.1% (v/v) 2-mercaptoethanol, 0.001% (w/v) bromophenol blue, 0.063 M-tris–HCl pH 6.8]. Slices were rinsed in equilibration buffer lacking 2-mercaptoethanol before loading into stacking gel sample wells which already contained 3 µg V8 protease set in 0.3% (w/v) agarose, 0.1% (w/v) SDS, 0.001% bromophenol blue, 0.063 M-tris–HCl pH 6.8. Peptides were then generated and analysed by one of two alternative procedures. Firstly, the procedure of Cleveland et al. (1977) was used. Electrophoresis was at 40 mA/gel until the dye front reached the interface of stacking gel and resolving gel (17% polyacrylamide). The gel apparatus was then filled with water prewarmed to 37 °C and incubated at 37 °C for 30 min. After incubation was complete, the current through the apparatus was restarted and the gel was run with an ample supply of cooling water until the dye front was within 1 cm of the gel end. Secondly, the electrophoresis was at 15 W/gel constant power until the dye front was 5 mm from the interface of stacking gel and resolving gel [10% polyacrylamide containing 50% (w/v) sucrose]. The current was then stopped, the apparatus was filled with prewarmed water and incubated as above. After incubation, electrophoresis was restarted and continued until the dye front was within 1 cm of the gel end. In either case, the gel was stained with Coomassie Brilliant Blue, then processed for fluorography.

The 10% polyacrylamide:sucrose gel system allows resolution of a wide range of polypeptide mol. wt., from above 95K to below 6K with less risk of gels cracking during drying than when using high polyacrylamide concentrations (above 10%). Normal Laemmli buffers were used throughout, and sucrose was dissolved in the separating gel mix before
polymerization. One μl of $N,N,N',N'$-tetramethylethylenediamine (TEMED) was used to catalyse polymerization of 25 ml of gel mix, rather than the normal 10 μl used for 25 ml of SDS–PAGE gel mix. Digestion within the stacking gel rather than at the stacking gel resolving gel interface gave more extensive digestion of the substrate polypeptide by V8 protease under the conditions described.

RESULTS

Unless noted otherwise in the text all experiments were performed with NDV strain Beaudette C. The base to acid orientation is left to right in all two-dimensional fluorographs shown. NEPHGE gels for Fig. 1 and 6 contained 2% ampholines pH range 3.5 to 10. IEF gels for Fig. 4 and 5 contained 1% ampholines pH range 3.5 to 10, 1% ampholines pH range 5 to 7.

Investigation of NDV primary translation products

NDV-infected CEF were labelled, and polypeptide synthesis was investigated, to determine which NDV-induced non-structural polypeptides were primary translation products, and which might be secondary products produced by proteolysis or other modification of the primary products which can change the SDS–PAGE mobility.

If the non-structural polypeptides 36K, gp62, and gp40 are primary translation products, rather than secondary products, the quantity relative to other primary products (HN, F₀, NAP, NP and M) should be high in a short pulse-labelling experiment. Furthermore, if all primary translation products are metabolically stable, their relative quantities will not change in a longer pulse labelling. If any polypeptide is generated by modification of a primary product, it might be absent from a short pulse but appear in a longer pulse or in a pulse–chase. For example, F₀ can be chased into F₁ in a pulse–chase (Samson & Fox, 1973; Nagai et al., 1976 a) since F₀ is the primary product and F₁ is a secondary cleavage product.

Pulse and pulse–chase experiments were performed on NDV-infected cells 8 h post-infection, and the resulting two-dimensional fluorographs are shown in Fig. 1. A 5 min pulse is shown in (a), a 1 h pulse in (b) and a 1 h pulse followed by a 1 h chase in (c). As expected, F₀ was cleaved to F₁ between (b) and (c). 36K was present in equal amounts in (a) and (b) which is the behaviour expected from a metabolically stable primary translation product. In (c), there was an increase in the more acidic, phosphorylated form of 36K (Chambers & Samson, 1980). On the other hand, gp40 showed labelling kinetics similar to F₁, being absent from (a), with a trace appearing in (b), and maximal in (c). Thus, it seems likely that gp40 is a secondary product formed by some modification or cleavage of one of the primary virus polypeptides. HN charge heterogeneity increased between (a) and (b), indicating a slow processing and the relative amount of HN declined between (b) and (c). These experiments indicate that 36K is a primary translation product, whereas gp40 is not. Extremely rapid processing of a larger virus polypeptide, if complete within 5 min, could also account for the presence of 36K.

An unexpected finding was that a highly basic polypeptide of mol. wt. 33K was present in (a), declined in (b), and disappeared from (c). This polypeptide was not seen in uninfected CEF and behaved like a metabolically unstable primary virus translation product, following the arguments above. Polypeptide 33K was not found in virions (not shown). Vertical arrows in Fig. 1 indicate host polypeptides which disappeared during chase incubation. Only the most acidic of these polypeptides was secreted into the culture medium, so the others may be degraded.

Comparison of the partial digestion products of NDV polypeptides

Partial digest peptide analysis of 36K, gp40 and gp62 was performed to determine whether their partial digestion patterns resembled those of the major virus polypeptides HN, F, NP,
Fig. 1. Fluorographs of two-dimensional polyacrylamide gel separations of NDV-infected CEF labelled with [14C]leucine at 8 h post-infection. (a) Pulse-labelled for 5 min; (b) pulse-labelled for 1 h; (c) pulse-labelled for 1 h, followed by a 1 h chase in medium containing 100 µg/ml unradioactive leucine. NDV-induced polypeptides HN, F₀, NAP, NP, M and 36K were present in all separations. 33K was detectable only in (a) and (b), gp40 only in (b) and (c). F₁ only in (c). Three host polypeptides which disappeared during chase incubation are indicated with vertical arrows, and the stable host polypeptide actin is indicated as A. Spot intensities were matched by exposing (a) for 36 days; (b) and (c) for 3 days.

NAP and M. A fairly high level of radioactivity in the various polypeptides is needed for partial digestion analysis, and this can be obtained by labelling infected cells for 3 h.

A 3 h pulse gave a pattern of spot intensities intermediate between that in Fig. 1 (b) and Fig. 1 (c), and since 33K is metabolically unstable, insufficient label was incorporated for analysis of this polypeptide. Results are shown in Fig. 2. The upper part shows peptide analysis on a
Fig. 2. Fluorographs of the partial digest peptides of [14C]leucine-labelled polypeptides from NDV-infected CEF. The upper part shows peptides analysed on a 17% polyacrylamide gel. The lower part shows peptides analysed on a 10% polyacrylamide:sucrose gel. The peptides from HN, F₀, gp62, F₁, gp40, M, NP, NAP and 36K are indicated beneath the gels. gp62 and F₁ were not analysed on the lower gel. An indication of peptide mol. wt. (× 10⁻³) is given on the right of the figure.

Fig. 3. Fluorographs of SDS-PAGE separations of (a) [14C]leucine-labelled purified NDV; (b) [3H]glucosamine-labelled NDV; (c, d) [3H]glucosamine-labelled CEF infected with NDV strains Beaudette C (c) or HP16 (d). The virion polypeptides L, HN, NP, A (actin derived from previous host cells), M and F₂ are indicated at the left of the figure. The NP band also contained NAP and F₁. The virion and intracellular glycosylated polypeptides HN, F₀, F₁, gp40 and F₂ are indicated on the right of the figure. In all tracks, F₂ is the major labelled polypeptide migrating at the dye front.

17% polyacrylamide gel; the lower part shows analysis on a 10% polyacrylamide:sucrose gel. The upper part demonstrates that F₀ and F₁ generated very similar peptides, consistent with the known precursor–product relationship between them. When present in sufficient quantity for analysis, gp62 generated similar peptides to HN. Although gp40 also generated similar peptides to HN, the relationship derived from the upper gel alone is not conclusive. M, NP, NAP and 36K generated dissimilar peptides. The lower gel, a more extensive digestion, now shows a clear relationship between HN and gp40, but reveals no more obvious similarities in the other polypeptides.

Partial digest peptide analysis suggests that gp62 and gp40 are related to HN. Pulse–chase experiments showed that HN declined and gp40 increased during a chase, and it therefore seems likely that gp40 is a major breakdown product of HN of unknown significance. While partial digest peptide analysis does not suggest that 36K is related to any of the major virus polypeptides (consistent with the short pulse-labelling data of Fig. 1) these results are not
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Fig. 4. Segments of two-dimensional fluorographs containing [14C]leucine-labelled CEF infected with NDV strains (a) Beaudette C, (b) LK, (c) Texas, (d) AV, (e) HP16, (f) B1, (g) N and (h) F. NDV-induced polypeptides HN, F₀, NAP, F₁ and 36K are indicated. F₀ was not detectable in (b); F₁ was not detectable in (f), (g) or (h). Host cell actin (A) is indicated in all segments.

sufficient to conclude that 36K is unique. The minor L polypeptide was not analysed because it was not resolved.

**Presence of gp40 in NDV-infected cells**

Experiments were performed to determine whether gp40 was present in CEF infected with strains of NDV other than Beaudette C, and to determine whether any gp40 appears in virions.

In Fig. 3, [14C]leucine-labelled virion polypeptides of NDV strain Beaudette C (a) are compared with [3H]glucosamine-labelled polypeptides from virions (b) or CEF infected with NDV strains Beaudette C (c) or HP16 (d). The important point is that a prominent gp40 band can be seen in Beaudette C-infected CEF (c), but not in (a), (b) or (d). Thus, gp40 is non-structural in strain Beaudette C, and not present in CEF infected with NDV strain HP16. Cells infected with most strains of NDV contain a small amount of gp40, more readily detectable by two-dimensional electrophoresis of [3H]glucosamine-labelled extracts. Of strains studied here, only HP16 and AV did not generate any gp40, and Beaudette C generated the largest amount (results not shown); gp40 could also be detected in NDV (Beaudette C)-infected BHK cells.

**Polypeptide variation in NDV-infected cells**

Strain-dependent variation in mol. wt. and pI of NAP in NDV-infected CEF and BHK cells was used as evidence that NAP is virus-coded (Chambers & Samson, 1980). NAP is not, however, the only NDV polypeptide that exhibits strain-dependent variation. This phenomenon is illustrated in Fig. 4, which shows CEF infected with several strains of NDV. L, NP and M polypeptides were insoluble in the IEF first dimension and, therefore, do not feature in the regions of the fluorographs shown.

HN and F₀ were identified on the basis of mol. wt. and glycosylation. NAP and 36K were identified on the basis of mol. wt. and phosphorylation. F₁ was identified either in pulse-chase experiments or by its mol. wt. and glycosylation. HN, F₀, F₁, NAP and 36K all exhibited strain-dependent variation, and it is clear that a version of the 36K polypeptide is synthesized in all NDV-infected monolayers. It is possible to group certain strains together on the basis of
the general similarity in pI of the virus polypeptides. For example, in Fig. 4 strains Beaudette C (a), LK (b) and Texas (c) had NAP and 36K of identical pI. F1 of Beaudette C and LK were identical (F0 of LK was not detected, as it is rapidly cleaved to F1; Samson & Fox, 1973) but were more acidic than F1 of Texas. HN of LK and Texas were identical, but were more acidic than HN of Beaudette C. All polypeptides of the Beaudette C, LK, Texas group differed from those of AV (d), but 36K, F0 and F1 of AV were similar to those of HP16 (e). Strains B1 (f), N (g) and F (h) showed no close resemblance to each other or to the strains in (a to e). F0 is not cleaved to F1 in CEF infected with these (B1, N and F) avirulent strains of NDV (Nagai et al., 1976a). Thus, it appears that NDV strains can be identified on the basis of the pI of polypeptides synthesized in infected CEF. 36K appears to be a useful strain marker, as it is more soluble than L, NP or M.

We reported earlier that synthesis of 36K was unaffected by 12 h incubation with actinomycin D, sufficient to cause a considerable reduction in the rate of synthesis of all host polypeptides. This indicated that 36K is virus-coded. A further test of the virus coding of 36K is to determine whether the strain-dependent variation of 36K shown in CEF (Fig. 4) is reproduced in BHK cells, as was shown for NAP. Sections of two-dimensional fluorographs are shown in Fig. 5. (a to d) are from CEF cells; (e to h) from BHK cells. (a, e) are uninfected; (b to d) and (f to h) are infected with NDV strains Beaudette C (b, f), N (c, g) or HP16 (d, h). Actin (A) is similar in CEF and BHK cells, and provides a useful reference point. It is clear that the variation in pI of 36K (Beaudette C > N > HP16) seen in CEF is exactly reproduced in BHK cells, confirming that 36K is virus-coded.

The synthesis of the 33K polypeptide was next investigated, and the results are shown in Fig. 6. Since 33K had not been detected in many previous 3 h duration labellings a
Fig. 6. Segments of two-dimensional fluorographs containing [14C]leucine-labelled 33K induced by three strains of NDV. (a to e) CEF cells; (f to i) BHK cells. (a, f) Uninfected; (b to e) and (g to i) infected with NDV strains Beaudette C (b, g), N (c, h) or HP16 (d, e, i). (e) was treated with actinomycin D throughout the post-infection incubation and labelling period. 33K spots are indicated with oblique arrows. A host polypeptide is indicated with a horizontal arrow in all figures as a reference point. Because the 33K spots are of low intensity, a tracing is included to show the spot positions more clearly. Host polypeptides are indicated as open spots; 33K as filled spots. The same host and 33K spots are indicated with arrows as in the photograph.

pulse-labelling protocol was employed. With the benefit of hindsight, however, it is possible to detect a very faint trace of 33K in our previous experiments. Relevant sections of fluorographs are shown in Fig. 6, labelled for 10 min at 8 h post-infection. (a to e) are from CEF cells; (f to i) are from BHK cells. (a, f) are uninfected; (b to e) and (g to i) are infected with NDV strains Beaudette C (b, g), N (c, h) or HP16 (d, e, i). At this extreme basic end of the NEPHGE gel, it is difficult to precisely reproduce variation from gel to gel. Fortunately, the version of 33K
induced by strain N (c, h) has a higher SDS mobility than those from strains Beaudette C (b, g) or HP16 (d, i). Strain variation in mol. wt. was reproducible, although the most basic form of 33K of HP16 in BHK (i) had smeared and showed a slightly different position to that seen in Fig. 6 (d) and (e). Fig. 6 (e) showed that 33K synthesis was unaffected by the presence of actinomycin D, whereas the synthesis of host polypeptides was much reduced. 33K of strain HP16 shows charge heterogeneity (it was assumed that both spots are forms of the same polypeptide). Charge heterogeneity of 33K of strain Beaudette C was less marked, and 33K of strain N showed no detectable charge heterogeneity.

These experiments showed that 33K is not synthesized in uninfected cells, shows strain-dependent variation in mol. wt. and its synthesis is unaffected by actinomycin D. These data are analogous to those presented for 36K and previously for NAP (Chambers & Samson, 1980) and suggest that 33K is also virus-coded. Peptide fingerprint data on 33K are not yet available for the reasons described earlier, so there is only kinetic evidence to show that 33K is a primary translation product.

**DISCUSSION**

The data presented here demonstrate that gp40 and gp62 are breakdown products of the HN polypeptide, but suggest that 36K and 33K are primary translation products. Thus, 36K and 33K should be considered as genuine virus non-structural polypeptides. Rima et al. (1980) detected two non-structural polypeptides in mumps virus-infected cells, mol. wt. 23K (C) and 17K (S). A metabolically unstable non-structural polypeptide (mol. wt. 15K to 18K) has been detected in measles virus-infected cells and canine distemper virus-infected cells (Hall et al., 1980; Rima & Martin, 1979; Campbell et al., 1980). Preliminary results with Sendai virus-infected cells (P. Chambers, unpublished results) show the presence of two non-structural polypeptides in both CEF and BHK cells. One is the previously described C/C' complex; the other has a mol. wt. of 56K and is unstable in chase incubations. Synthesis of both Sendai non-structural polypeptides is unaffected by actinomycin D. C/C' shows strain variation (Etkind et al., 1980) but strain variation of 56K has not yet been investigated. Two non-structural polypeptides may be a general feature of paramyxoviruses, with one metabolically stable and the other unstable.

The role of these non-structural polypeptides is still a matter for speculation. Since paramyxovirus virion nucleocapsids contain the various enzymes necessary for transcription (Huang et al., 1971; Weiss & Bratt, 1976; Colonno & Stone, 1975, 1976a, b) but do not contain either 36K or 33K, it seems that virus transcription does not require these polypeptides. Replicative processes, such as synthesis of full length positive and negative strands of RNA are not performed by isolated virion nucleocapsids, and perhaps require the participation of non-structural polypeptides. Virus assembly and budding processes are other possible sites of action. One way to determine the role of non-structural polypeptides in replication and assembly is to identify mutants with a lesion in a particular polypeptide showing an electrophoretic shift, and to correlate this physical variable with a biological function (King & Newman, 1980; Samson et al., 1981). A second approach is to examine the kinetics of nucleocapsid and virus assembly, and the polypeptides associated with nucleocapsids and cellular fractions (Portner & Kingsbury, 1976; Nagai et al., 1976b; Lamb & Choppin, 1977; Smith & Hightower, 1981b).

Both 36K and 33K show charge heterogeneity. That of 36K is due to phosphorylation, but the cause of that of 33K is not yet known. Rapid disappearance of 33K in pulse-chase experiments, and charge heterogeneity, increase the possibility of metabolic control exerted by the non-structural polypeptides.

Not all strains of NDV generate gp40, so there may not be a biological role for this polypeptide. Pulse-chase experiments with several strains of NDV (Beaudette C, Texas, N,
AV, HP16) show that where HN is stable in a chase, no gp40 appears (AV, HP16), but that where HN is unstable in a chase gp40 can be detected (Beaudette C, N, Texas). The gp40 itself disappears in a 4 h chase, and may be more stable in strain Beaudette C than in the other strains in which it can be detected. Since half of the HN molecule is basic (gp40), the other half must be acidic, as the HN polypeptide itself has a neutral pI. Salt-shock mapping could be used to determine which part of the HN molecule corresponds to gp40 (Samson et al., 1980) and since inspection of fluorographs suggests that gp40 contains a disproportionate amount of the HN carbohydrate, a rough map of the HN molecule could be drawn up, showing regions rich in acidic amino acids, basic amino acids and carbohydrate. Thus, continued study of gp40 could yield interesting information about the biochemical structure of the HN molecules of paramyxoviruses. The disulphide-linked fragments of HN do not appear in two-dimensional gels, possibly lying outside the pI range studied (Samson et al., 1980).

Small mRNAs which may code for 36K and 33K have not yet been detected (Weiss & Bratt, 1976; Thomas et al., 1978). The positions of 36K and 33K in the genetic map of NDV remain to be determined, but Collins et al. (1980) found that the u.v. target size increment of the HN gene was large enough to encode a small additional polypeptide.

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