Frog Virus 3 Replication: Analysis of Structural and Nonstructural Polypeptides in Infected BHK Cells by Acidic and Basic Two-Dimensional Gel Electrophoresis

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Analysis of frog virus 3-infected BHK cells by two-dimensional, acidic and basic gel electrophoresis showed that at least 90 infected cell-specific polypeptides could be detected. These polypeptides represent between 70 and 85% of the coding capacity of the viral genome. The polypeptides were sequentially induced in at least three phases. The virus gradually suppressed host cell polypeptide synthesis during infection, although the synthesis of a few cell polypeptides may be "switched off" early in infection.

Frog virus 3 (FV3) is a large icosahedral DNA-containing virus which replicates in the cytoplasm but requires a functional cell nucleus for replication to occur (8, 9, 11). The virus possesses a linear double-stranded DNA genome having a molecular weight of about 10^8 (10) and so has the potential to synthesize about 100 polypeptides of average size. Recent studies in our laboratory with sensitive high-resolution one-dimensional gel electrophoresis resolved 29 infected cell-specific polypeptides (ICPs), of which 22 comigrated with virus structural polypeptides. The ICPs appeared to be coordinately regulated in a cascade fashion, with three temporal phases (α , β , and γ) being observed (6). The synthesis of γ polypeptides requires synthesis of viral progeny DNA to occur (5, 6). In baby hamster kidney (BHK 21/13) cells the α phase polypeptides are detected from 2 h after infection, the β polypeptides are detected from 4 h, and the γ -phase polypeptides are detected from 6 h. The polypeptides detected represent 30% of the coding capacity of the viral genome.

The development of two-dimensional gel electrophoretic techniques to resolve proteins by separating proteins in one dimension according to charge with isoelectric focusing (IEF) and then separating them in the second dimension according to size with conventional electrophoresis in the presence of sodium dodecyl sulfate (SDS) and reducing agents has produced a powerful tool to explore complex proteins (13, 14). In this research we used this methodology to resolve virus-induced polypeptides in FV3-in-

fect cells and so examine the number of polypeptides present in infected cells, to determine the effect of the virus on host cell polypeptides, and also to confirm the temporal induction of FV3 polypeptides.

MATERIALS AND METHODS

Materials. L-[³⁵S]methionine (840 Ci/mmol) and ¹²⁵I (100 mCi/ml) were purchased from the Radiochemical Centre, Amersham, England. Ampholines were purchased from L.K.B. Ltd., Croydon, England. DNase I and RNase A were obtained from the Sigma Chemical Co. Ltd., London, England.

Cells and virus. Baby hamster kidney (BHK 21/13) cells and fathead minnow (FHM) cells were grown and maintained as previously described, and FV3 was grown and titrated in FHM cells as previously described (4).

Radiolabeling of cellular polypeptides with [³⁵S]methionine. BHK cells were grown in small plastic petri dishes (30 mm; 3×10^6 cells) at 37°C overnight. Cells were infected at 25 to 30 PFU of FV3 per cell by adsorbing at room temperature for 1 h and then washing with the Glasgow modification of Eagle minimal essential medium. The infection was allowed to proceed at 28°C. Cells were pretreated with radiolabeling medium (2% dialyzed calf serum in Earle balanced salt solution) for 15 min before being pulsed with 100 μ Ci of L-[³⁵S]methionine (in 1 ml) for 1 h at 28°C. The isotope was removed, and the cells were washed three times in ice-cold phosphate-buffered saline and then pelleted. The cells were resuspended in 100 μ l of 10 mM Tris-hydrochloride (pH 7.4)–50 mM NaCl–5 mM MgCl₂ containing 25 μ g of DNase I per ml and 25 μ g of RNase A per ml. The cells were sonicated and then incubated on ice for 30 min. The cells were immediately freeze dried.

Two-dimensional gel electrophoresis of polypeptides. The procedures followed were essentially those derived by O'Farrell and co-workers (13, 14).

Freeze-dried cells were resuspended in 100 μ l of lysis buffer (9.4 M urea, 2% Nonidet P-40, 1.6% ampholines

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[pH 5 to 7], 0.4% ampholines [pH 3.5 to 10], 5% β -mercaptoethanol, and 250,000 cpm of material (about 20 μ l) was applied to each electrofocusing gel.

The first-dimension separations were performed either as (i) acidic 4% polyacrylamide gels (130 by 2.5 mm) containing 2% ampholines (1.6% pH 5 to 7, 0.4% pH 3.5 to 10) or (ii) basic nonequilibrium pH gradient electrophoresis (NEPHGE) 4% polyacrylamide gels (130 by 2.5 mm) containing 2% ampholines (1% pH 7 to 9, 1% pH 8 to 9.5) exactly as described by O'Farrell and co-workers (13, 14). Both IEF and NEPHGE gels were then immersed in equilibrium buffer (0.06 Tris, pH 6.8, 4% SDS, 10% β -mercaptoethanol, 20% glycerol) and incubated at room temperature for 30 min before being applied to the second SDS-polyacrylamide dimension. Gels to be stored frozen were incubated for 15 min only, and the incubation was completed after defrosting.

The second-dimension separations by SDS-polyacrylamide gel electrophoresis of the proteins contained in the IEF gels were accomplished by layering the equilibrated gels onto a stacking gel (4.75% acrylamide, 0.024% bisacrylamide, 0.1% SDS, 0.0625 M Tris-hydrochloride, pH 6.8) which overlaid a 15% acrylamide gel (0.1% SDS, 0.375 M Tris-hydrochloride, pH 8.8). A 1% agarose gel made up in electrophoresis buffer and containing 0.01% bromophenol blue secured the gel in place. The electrophoresis buffer contained 0.025 M Tris, 0.192 M glycine, and 0.1% SDS, pH 8.8. Electrophoresis was performed at 6 to 10 mA for 14 to 18 h. The slab measured 19 by 16 cm. The gels were processed for fluorography (2) by being fixed in 45% methanol-7.5% acetic acid-47.5% water for 1 h, incorporating PPO (2,5-diphenyloxazole), drying, and then being exposed to Fuji X-ray film as described in an accompanying paper (6).

The isoelectric points of individual polypeptides are given to the nearest 0.01 pH unit for convenience of identification. The values were obtained by extrapolation and do not represent measurements made to the nearest 0.01 pH unit.

One-dimensional SDS-polyacrylamide gel electrophoresis. This was based on the second dimension described above and followed procedures described in an accompanying paper (6), except that the reservoir buffer was twice concentrated and the acrylamide-bisacrylamide ratio was increased to 200:1 from 75:1; 15% acrylamide gels were run. The samples contained ampholines as described for the two-dimensional work and were mixed with an equal volume of 10% SDS-10% β -mercaptoethanol-15% glycerol-0.02% bromophenol blue in 1 M Tris-hydrochloride, pH 6.8.

Iodination of FV3 particles with 125 I using chloramine T. This was performed exactly as described by Moore et al. (12).

RESULTS

Incorporation of [35 S]methionine into trichloroacetic acid-insoluble material. It has been claimed that FV3 is a potent inhibitor of protein synthesis in cells infected with live or inactivated virus (3). We were unable to confirm these observations since the incorporation of [35 S]methionine into trichloroacetic acid-insoluble

material was at an equivalent or higher level than control cells for most of the infection (Table 1). The data reported indicate the results obtained with material run on both the one-dimensional and the two-dimensional gels. Similar results have been obtained in numerous other experiments.

One-dimensional SDS-polyacrylamide gel electrophoresis of polypeptides in FV3-infected BHK cells. The sequential induction of FV3 ICPs on a gel system identical to the two-dimensional gel electrophoretic second dimension is shown in Fig. 1; α polypeptides were detected from 2 h after infection, β polypeptides were detected from 4 h, and the onset of γ synthesis was at 6 h. In this particular experiment the β and γ phases were slightly late, so the β polypeptides predominated at 6 h and the γ polypeptides, which were barely detected at 6 h, predominated later. Basically the same pattern of polypeptides was observed on those gels as on our standard SDS-polyacrylamide gel electrophoresis gels (6), although two additional bands are detected in purified virus (polypeptides X and 88) which are not normally detected and X is not present as an ICP. There are also "anomalies" in the running of certain polypeptides in the molecular weight range of 3×10^4 to 6×10^4 . In these experiments the acrylamide-bisacrylamide ratio was different than that used for our standard conditions (6), and the samples contained 4 M urea and 0.5% ampholines, so some anomalies may have been due to these differences. The ICPs are labeled according to the nomenclature used in an accompanying paper (6).

Nomenclature for two-dimensional ICPs. The ICPs detected by SDS-IEF and SDS-

TABLE 1. Incorporation of L- 35 S]methionine into trichloroacetic acid-insoluble material in FV3-infected BHK cells at various times after infection

Sample	Time (h)	Trichloroacetic acid-insoluble counts ^a
Control		45,529
FV3	2	55,018
FV3	4	41,647
FV3	6	54,543
FV3	9	44,879
FV3	12	42,748
FV3	24	46,997

^a Counts per minute obtained after precipitation of material in 6×10^4 cells infected at 25 to 30 PFU of virus per cell and pulse-radiolabeled with 5 μ Ci of L- 35 S]methionine for 1 h. Precipitation was achieved by adding an equal volume of ice-cold 20% trichloroacetic acid and incubating on ice for 60 min. The control was uninfected cells held at the infection temperature (28°C).

FIG. 1. Sequential induction of ICPs in BHK cells infected with FV3. Cells were pulse-labeled with [35 S]methionine for 1 h at the times indicated across the top of the figure (in hours). Purified FV3 was co-run to indicate structural polypeptides. ICPs are identified by estimates of their molecular weights ($\times 10^3$) (6) and are assigned as virus particle (VP) or nonstructural (NS). U, Uninfected.

NEPHGE are shown in Fig. 2 and cataloged in Table 2. The polypeptides are identified by a combination of molecular weight (to the nearest 1,000) and isoelectric point (to the nearest 0.01 pH unit), so that the major structural polypeptide of FV3 is 58,5.58 and the major nonstructural basic polypeptide is 35,7-10. The pH range is indicated for basic polypeptides because nonequilibrium electrophoresis was performed (14).

The various polypeptides were designated α , β , and γ for comparison with one-dimensional gel polypeptides (6). The assignment was not rigorous because the requisite "function-blocking" experiments had not been performed yet (6). For the purpose of this paper, the timing and apparent amount of synthesis were used for assignment; α polypeptides were synthesized from 2 h (although apparent isomers of polypeptide 16,5.48 were detected from 4 h), β poly-

peptides were detected at 4 or 6 h and subsequently synthesized in similar amounts, and γ polypeptides were synthesized at 6 or 9 h and predominated late in infection.

Acid IEF and SDS-acrylamide gel electrophoresis of FV3 ICPs. Uninfected BHK cells contained well in excess of 200 polypeptides resolved by SDS-IEF (Fig. 3, gel i). By 2 h after infection three ICPs were detected, all of which were nonstructural polypeptides (Fig. 3, gel ii). These three polypeptides (ICP 46,5.55, ICP 45,5.47, ICP 16,5.48) are by definition α polypeptides. Most cell polypeptides continued to be synthesized, albeit at a reduced rate, and some major cell polypeptides were markedly reduced. At 4 h after infection these three α polypeptides were synthesized in large amounts, and an additional four presumptive isomers of polypeptide 16,5.48 were detected (Fig. 3, gel iii and Table 2). Seven additional polypeptides, all presump-

FIG. 2. Diagrammatic representation of ICPs detected on (A) SDS-IEF and (B) SDS-NEPHGE two-dimensional gels. ICPs tentatively assigned as structural polypeptides are shown as closed areas, and nonstructural polypeptides are shown as open areas or are indicated NS.

TABLE 2. *Catalog of ICPs detected in FV3-infected cells by two-dimensional gel electrophoresis*

Polypeptide	Structural or nonstructural ^a	Period detected (h)	α , β , or γ	Equivalent one-dimensional gel polypeptide	Polypeptide	Structural or nonstructural ^a	Period detected (h)	α , β , or γ	Equivalent one-dimensional gel polypeptide
Acidic									
86,5.76 ^b	NS	9-24	γ	ICP 76 γ VP or ICP 78 γ VP	29,4.14	NS	9-24	γ	ICP 27 γ VP
82,5.76	NS	9-24	γ		29,4.18	S?	9-24	γ	
76,6.85	S	9-24	γ		29,4.46	S	6-24	β	ICP 29 β VP
					27/28,4.50	NS	4-9	β	ICP 23 β NS
					24,4.76	NS	6-24	β	
72,5.53	NS	9-24	γ	ICP 69 β VP	24,4.88	NS	6-24	β	ICP 23 β NS
72,5.63	S	6-24	γ		24,6.37	NS	6-24	β	
72,5.73	NS	9-24	γ		23,6.46	NS	6-24	β	ICP 23 β NS
69,6.93	S	4-24	β		22,4.76	NS	6-24	β	
59,5.93	NS	9-24	γ		22,4.88	NS	6-24	β	ICP 22 γ NS
59,6.11	NS	9-24	γ	ICP 55 γ VP	22,5.23	NS	6-24	γ	
59,6.22	NS	9-24	γ		22,6.53	NS	6-24	β	ICP 16 α NS
59,6.35	S	6-24	γ		18,5.91	NS	6-24	β	
59,6.51	NS	9-24	γ		17,7.00	NS	6-24	β	ICP 12 γ VP
58,5.41	S	6-24	γ		16,4.93	NS	4-24	α	
58,5.48	S	9-24	γ	ICP 52 β VP	16,5.22	NS	4-24	α	ICP 114 γ VP
58,5.58	S	6-24	γ		16,5.30	NS	4-24	α	
58,5.69	S	9-24	γ		16,5.48	NS	2-24	α	ICP 98 β VP
58,6.99	NS	9-24	γ		16,5.70	NS	4-24	α	
55,5.39	NS	6-24	γ		16,7.00	S	6-24	β	ICP 66 β NS
55,6.93	NS	4-24	β	ICP 40 γ NS ICP 37 γ VP	15,4.86	NS	4-9	β	
54,5.82	NS	9-24	γ		15,6.52	NS	4-24	β	ICP 44 β VP?
54,5.94	NS	9-24	γ		15,6.80	NS	6-24	γ	
54,6.12	NS	9-24	γ		14,4.14	NS	9-24	γ	ICP 35 β NS ^d
52,5.39	S	6-24	β		14,4.57	S	6-24	γ	
49,4.76	NS	6-9	β	ICP 46 β VP	13,6.25	S	12-24	γ	ICP 12 γ VP
49,5.13	S	12-24	γ		Basic				
49,5.23	S	12-24	γ		115,7-10 ^c	S	9-24	γ	ICP 98 β VP
49,4.35	NS	12-24	γ		114,7-10	NS	12-24	γ	
47,5.45	NS	4-12	β		100,7-10	S	6-24	β	ICP 66 β NS
46,5.55	NS	2-24	α	ICP 40 γ NS ICP 37 γ VP	100,7-10bi	S	6-24	β	
46,5.63	NS	4-?	β		100,7-10bii	S	6-24	β	ICP 44 β VP?
45,5.47	NS	2-24	α		100,7-10biii	S	6-24	β	
43,5.60	NS	12-24	γ		76,7-10	NS	9-24	γ	ICP 35 β NS ^d
41,5.57	NS	12-24	γ		67,7-10	NS	4-24	β	
38,5.30	S	9-24	γ	ICP 37 γ VP	55,7-10	NS	9-24	γ	ICP 12 γ VP
38,5.56	NS	12-24	γ		47,7-10	NS	9-24	γ	
35,4.57	NS	6-24	β		38,7-10	S	6-24	β	ICP 12 γ VP
34,5.56	NS	12-24	γ		38,7-10b	NS	4-24	β	
34,6.56	S	6-24	γ		35,7-10	NS	4-24	β	ICP 12 γ VP
34,6.70	NS	6-9	β	ICP 37 γ VP	24,7-10	NS	9-24	γ	
33,5.35	NS	12-24	γ		24,7-10b	S	6-24	β	ICP 12 γ VP
33,5.65	NS	12-24	γ		18,7-10	S	9-24	γ	
32,5.53	NS	12-24	γ		15,7-10	S	9-24	γ	ICP 12 γ VP
31,4.64	NS	9-24	γ		14,7-10	NS	12-24	γ	
31,5.35	NS	9-24	γ		13,7-10	S	9-24	γ	ICP 12 γ VP
					12,7-10	S	6-24	γ	

^a S, Structural; NS, nonstructural. The brackets on the table indicate apparent charge isomers.^b For the acidic polypeptides the first number is the molecular weight ($\times 10^3$), and the second number is the isoelectric point (pH).^c For the basic polypeptides the first number is the molecular weight ($\times 10^3$), and the second number is the pH range resolved.^d See text.

tive β polypeptides, were observed, including the structural polypeptide 69,6.93. Little further suppression of cellular synthesis occurred. By 6 h β polypeptide synthesis was well under way, and an additional 15 β ICPs were detected (Fig. 3, gel iv and Table 2). At 6 h eight γ polypeptides were detected, including the major virus structural polypeptide 58,5.58. By 9 h (Fig. 3, gel v) these polypeptides became the prominent γ polypeptides synthesized, although at this time

a number of additional γ polypeptides were detected (Table 2). Minor γ ICPs were detected at 12 and 24 h (Fig. 3, gels vi and vii). At 24 h after infection, a number of β and γ polypeptides had declined in synthesis, although the main virus structural polypeptides were still synthesized in quantity (Fig. 3, gel vii). About 70 ICPs were resolved during infection.

The structural polypeptides contained in FV3 and resolved by SDS-IEF are shown in Fig. 3,

FIG. 3. Distribution of [^{35}S]methionine-labeled polypeptides in FV3-infected BHK cells analyzed by SDS-IEF. Gel i, Uninfected cells radiolabeled for 1 h at 28°C; gel ii, infected cells radiolabeled at 2 to 3 h; gel iii, infected cells radiolabeled at 4 to 5 h; gel iv, infected cells radiolabeled at 6 to 7 h; gel v, infected cells radiolabeled at 9 to 10 h; gel vi, infected cells radiolabeled at 12 to 13 h; gel vii, infected cells radiolabeled at 24 to 25 h; gel viii, purified [^{35}S]methionine-labeled FV3 structural polypeptides. The ordinate represents molecular weight (MW) ($\times 10^3$).

gel viii. Most polypeptides detected in virus particles possessed ICP counterparts, although a few had no obvious ICP or cellular polypeptide counterpart. Virus particles presented consistent profiles, and so this discrepancy may be due either to enrichment of minor infected cell proteins in the virus particles or to processing on encapsidation and release.

Basic NEPHGE and SDS-acrylamide gel electrophoresis of FV3 ICPs. In contrast to the acidic system, few ICPs were detected in the basic pH 7 to pH 10 analysis. The 20 polypeptides detected are shown diagrammatically in Fig. 2 and sequentially in Fig. 4. A summary of their properties is given in Table 2.

No ICPs were detected 2 h after infection (Fig.

4, gel ii), and there was not a marked alteration in the synthesis of cell polypeptides, although the synthesis of certain cell polypeptides was obviously becoming suppressed. At 4 h the synthesis of three nonstructural β polypeptides (ICP 67,7-10; ICP 38,7-10b; ICP 35,7-10) was detected, and by 6 h an additional four structural β polypeptides were detected (Fig. 4, gels iii and iv), which increased in relative amount of synthesis at 9 h (Fig. 4, gel v). At 6 h one structural γ polypeptide (ICP 12,7-10) was detected. Subsequently at 12 and 24 h little alteration in ICP synthesis occurred, although cell polypeptide synthesis was further suppressed.

Identification of structural and nonstructural polypeptides by SDS-NEPHGE was a problem.

FIG. 3. v-viii

[³⁵S]methionine-labeled FV3 particles disrupted with lysis buffer or lysis buffer plus 0.1% SDS failed to produce reproducible patterns. Purified FV3 subsequently radiolabeled with ¹²⁵I using chloramine T demonstrated that three major polypeptides detected late in infection were basic structural polypeptides. Considerably more ¹²⁵I-labeled polypeptides were present in virus particles than were observed as ICPs in infected cells late in infection (and in SDS-NEPHGE resolving purified [³⁵S]methionine-labeled virus), and this we ascribe in part to chloramine T-induced charge alterations in the polypeptides and in part to differences in the methionine and tyrosine contents of the polypeptides. There is also the possibility of unlabeled contaminating host proteins becoming iodinated by chloramine T.

Comparison of SDS-IEF and SDS-NEPHGE. The ICPs detected over the pH range 4 to 10 are shown diagrammatically in Fig.

2 and in Fig. 5. A total of 70 acidic and 20 basic polypeptides were detected. The total molecular weight of these polypeptides was 3.89×10^6 , which represents 77% of the coding capacity of the FV3 genome (Table 3).

A number of ICPs detected by one-dimensional SDS-PAGE analysis appeared not to be detected by SDS-IEF and SDS-NEPHGE. These frog FV3 ICPs included ICPs 104, 93, 88, 61, 58, 18, and 9 (which are all minor α , β , and γ polypeptides which co-electrophorese with cellular polypeptides) and ICP 34, a major structural β polypeptide. With the exception of ICP 34, these polypeptides either may have an isoelectric point of pH 7.00 and so are not detected at the first-dimension gel origin (pH 7.0) above material which has not entered the gel or may have isoelectric points outside the pH range 4 to 10. The total molecular weight of the ICPs detected by two-dimensional gel analysis and the unresolved polypeptides (with the exception of

FIG. 4. Distribution of [^{35}S]methionine-labeled polypeptides in FV3-infected BHK cells analyzed by SDS-NEPHGE. Gel i, Uninfected cells radiolabeled for 1 h at 28°C; gel ii, infected cells radiolabeled at 2 to 3 h; gel iii, infected cells radiolabeled at 4 to 5 h; gel iv, infected cells radiolabeled at 6 to 7 h; gel v, infected cells radiolabeled at 9 to 10 h; gel vi, infected cells radiolabeled at 12 to 13 h; gel vii, infected cells radiolabeled at 24 to 25 h; gel viii, purified FV3 structural polypeptides ^{125}I iodinated using chloramine T. The ordinate represents molecular weight (MW) ($\times 10^6$).

ICP 34 [see below]) was 4.325×10^6 , which is equivalent to 85.6% of the virus genome (Table 3).

Effect of FV3 on cellular polypeptide synthesis. Over 400 polypeptides were detected in uninfected BHK cells and are too numerous to realistically and profitably catalog. At 24 h after infection 50 cell-specific polypeptides were detected. Since FV3 has no marked effect on total protein synthesis in BHK cells (Table 1), it is obvious that the virus subverts the capacity of the cell protein-synthesizing systems to synthesize its own virus-specific proteins. This appears to occur by suppressing cell protein synthesis rather than by actively switching off cell synthesis. Most cell polypeptides appear to become gradually undetectable with respect to time,

which is indicative of successful competition of virus synthesis with cell synthesis. Certain polypeptides do, however, decline rapidly in synthesis (e.g., polypeptides at position 60,5.01 and four polypeptides within the range 80 to 100,4 to 5).

DISCUSSION

Our results demonstrate that a high proportion of the FV3 genome appears to be transcribed and translated into presumptive virus-specific proteins during a productive infection. The results also show that two-dimensional gel electrophoretic analysis of FV3 ICPs is vastly superior to conventional one-dimensional analysis and that it could be the method of choice for the future analysis of FV3 polypeptide metabolism.

FIG. 4. v-viii

The results obtained on two-dimensional gels confirm that there are at least three temporal phases of polypeptide synthesis in FV3-infected BHK cells, substantiating our accompanying observations (6). This is at variance with the report by Granoff (9) that all FV3 ICPs are synthesized from the initiation of infection (although there are subsets of ICPs distinguished by their rate of synthesis [15]). Autoradiographic overexposure of the gels shown in this paper failed to demonstrate any of the β or γ ICPs at times earlier than those reported (unpublished data), and so we are confident that there was not a detection problem.

The separation of FV3 ICPs according to their isoelectric points in the IEF and NEPHGE dimensions allowed a catalog of isoelectric points to be compiled (Table 2), and this is particularly useful in the IEF separation. Our information shows that the bulk of the FV3 ICPs have isoelectric points in the pH 4 to 7 range, including

the major structural polypeptide (ICP 58,548).

Observations on the major structural polypeptide show that there is apparent isomerism of this polypeptide with polypeptides of different charges but similar molecular weights appearing in the cell. This polypeptide is phosphorylated but is not detectably sulfated or glycosylated (6), and so some isomerism may be attributed to phosphate-created charge differences. Not all phosphorylated ICPs show isomerism (e.g., ICP 69,693) and so some isomerism may be caused by as-yet-unidentified posttranslational modification. A number of other polypeptides appear to show charge isomerism and are shown in Table 2.

On one-dimensional gels ICP 34 (a major ICP) appears to be a structural β polypeptide, although on two-dimensional gels no counterpart is found. A comparison of SDS-IEF and SDS-NEPHGE indicates that a major β nonstructural polypeptide (ICP 34,7-10) is found on SDS-

FIG. 5. Distribution of total [^{35}S]methionine-labeled polypeptides in FV3-infected BHK cells; a comparison of SDS-IEF and SDS-NEPHGE at 4 and 12 h after infection. Gels i and iii, SDS-IEF and SDS-NEPHGE, respectively, 4 h; gel ii and iv, SDS-IEF and SDS-NEPHGE, respectively, 12 h. The ordinate represents molecular weight (MW) ($\times 10^3$).

NEPHGE gels from 4 to 24 h and that a minor γ structural polypeptide (ICP 34,6.56) occurs from 6 to 24 h on SDS-IEF gels; therefore, it appears that ICP 34 resolved on one-dimensional gels represents two polypeptides and that the structural component of the virus particle is an acidic γ polypeptide.

Early in infection no basic α polypeptides are detected, which may be considered surprising since the virus appears to have an effect on the expression of the cell genome, and the interaction of basic proteins with DNA or RNA or both may provide a mechanism of inhibition. The absence of proteins with basic isoelectric points does not preclude the possibility that some other

ICPs have basic regions capable of such interaction.

Our data indicate that reasonable quantities of FV3 (about 30 PFU/cell, 20 virus particles per PFU) fail to cause cell polypeptide synthesis to be switched off rapidly, although there is a gradual suppression of cell polypeptide synthesis as the infection progresses. This contradicts earlier published work (8), but is in agreement with observations of J. C. S. Clegg and S. I. T. Kennedy (personal communication) and R. W. Schlesinger (personal communication). FV3, like herpesvirus (7), undoubtedly possesses structural polypeptides which can inhibit cell RNA and protein synthesis, as elegantly shown by

TABLE 3. Total molecular weights of FV3-induced classes of ICPs detected by various gel analytical methods and the percentages of the FV3 genome required for their syntheses

Analytical method	Summed mol wt of the following ICP classes:				
	α^a	β^a	γ^a	S ^b	NS ^b
SDS-IEF	1.71×10^5 (3.4) ^c	6.85×10^5 (13.6)	1.933×10^6 (38.3)	8.31×10^5 (16.5)	1.958×10^6 (38.8)
SDS-IEF corrected for isomerism ^d	1.07×10^5 (2.1)	6.85×10^5 (13.6)	1.523×10^6 (30.1)	6.57×10^5 (13.0)	1.658×10^6 (32.8)
SDS-NEPHGE		6.02×10^5 (11.9)	5.03×10^5 (10.0)	6.35×10^5 (12.6)	4.70×10^5 (9.3)
SDS-NEPHGE corrected for isomerism		3.02×10^5 (6.0)	5.03×10^5 (10.0)	3.35×10^5 (6.6)	4.70×10^5 (9.3)
Unresolved ^e	6.1×10^4 (1.2)	2.48×10^5 (14.9)	1.22×10^5 (2.4)	3.25×10^5 (6.4)	1.06×10^5 (2.1)
Total ^f	2.32×10^5 (4.6)	1.535×10^6 (30.4)	2.558×10^6 (50.6)	1.791×10^6 (35.5)	2.534×10^6 (50.2)
Total corrected for isomerism	1.68×10^5 (3.3)	1.235×10^6 (24.5)	2.148×10^6 (42.5)	1.317×10^6 (26.1)	2.234×10^6 (44.2)

^a α , β , and γ refer to the temporal classes of ICPs resolved (6, 7).

^b S, Structural polypeptides; NS, nonstructural polypeptides.

^c The numbers in parentheses are the percentages of the FV3 genome (molecular weight, 1×10^6 [12]) responsible for the synthesis of individual classes of ICPs, assuming that the whole genome is available for translation and transcription and that no overlapping genes occur.

^d For corrections for possible isomerism, see text and Table 2.

^e Unresolved polypeptides are ICPs which are resolved on one-dimensional gels but have no two-dimensional counterparts (see text).

^f The total molecular weight for α plus β plus γ polypeptides was 4.325×10^6 or 85.6% of the FV3 genome (3.551×10^6 and 70.3% corrected for isomerism). For structural plus nonstructural polypeptides the molecular weight totals were the same as those for α plus β plus γ polypeptides.

Aubertin et al. (1), but it is doubtful that sufficient quantities of these proteins are introduced into cells under normal physiological conditions to suppress host polypeptide synthesis dramatically.

ACKNOWLEDGMENTS

We thank J. S. Knowland for his interest in these studies. R.M.E. received a Science Research Council postgraduate studentship. R.B. thanks the Comision Nacional de Investigacion Cientifica y Tecnologica de Chile, the Royal Society of London for an Exchange Fellowship, and the Medical Research Council for financial assistance.

LITERATURE CITED

- Aubertin, A. M., C. Hirth, C. Travo, H. Nonemacher, and A. Kirn. 1973. Preparation and properties of an inhibitory extract from frog virus 3 particles. *J. Virol.* **11**:694-701.
- Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**:83-88.
- Drillien, R., D. Sephner, and A. Kirn. 1977. Cell killing by frog virus 3: evidence for cell killing by single viral particles or single viral units. *Biochem. Biophys. Res. Commun.* **79**:105-111.
- Elliott, R. M., M. K. Arnold, and D. C. Kelly. 1979. The replication of frog virus 3 in an amphibian cell line (XTC-2) derived from *Xenopus laevis*. *J. Gen. Virol.* **44**:89-98.
- Elliott, R. M., A. Bateson, and D. C. Kelly. 1980. Phosphonoacetic acid inhibition of frog virus 3 replication. *J. Virol.* **33**:539-542.
- Elliott, R. M., and D. C. Kelly. 1980. Frog virus 3 replication: induction and intracellular distribution of polypeptides in infected cells. *J. Virol.* **33**:28-51.
- Fenwick, M. L., and M. J. Walker. 1978. Suppression and synthesis of cellular macromolecules by herpes simplex virus. *J. Gen. Virol.* **41**:37-51.
- Goorha, R., D. Willis, and A. Granoff. 1977. Macromolecular synthesis in cells infected with frog virus 3. VI. Frog virus 3 replication is dependent on the cell nucleus. *J. Virol.* **21**:802-805.
- Granoff, A. 1969. Viruses of amphibian. *Curr. Top. Microbiol. Immunol.* **50**:107-137.
- Kelly, D. C., and R. J. Avery. 1974. Frog virus 3 deoxyribonucleic acid. *J. Gen. Virol.* **24**:339-348.
- Kelly, D. C., and J. S. Robertson. 1973. Icosahedral cytoplasmic deoxyriboviruses. *J. Gen. Virol.* **20**:17-41.
- Moore, N. F., J. M. Kelley, and R. R. Wagner. 1974. Envelope proteins of vesicular stomatitis virions: accessibility to iodination. *Virology* **61**:292-296.
- O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**:4007-4021.
- O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell. 1977. High resolution two dimensional electrophoresis of basic as well as acidic proteins. *Cell* **12**:1133-1142.
- Willis, D. B., R. Goorha, M. Miles, and A. Granoff. 1977. Macromolecular synthesis in cells infected by frog virus 3. VII. Transcriptional and posttranscriptional regulation of virus gene expression. *J. Virol.* **24**:326-342.