Acta virol. 31 : 193—202, 1987

BIOLOGIC PROPERTIES AND GENOME STRUCTURE OF THE RECOMBINANTS BETWEEN ECTROMELIA AND RABBITPOX VIRUSES

V. I. CHERNOS, T. G. SENKEVICH, N. V. CHELYAPOV, T. P. ANTONOVA, T. S. VOVK, I. V. MITINA

Moscow Research Institute of Viral Preparations, U.S.S.R. Ministry of Health, 109 088 Moscow, U.S.S.R.

Received June 13, 1986; revised October 23, 1986

Summary. — Administration of rabbitpox virus (RPV) DNA, cleaved into 2 fragments by *Sma*I restrictase, into ectromelia virus (EMV)-infected chick fibroblast cells yielded recombinants whose properties were characteristic of both parents. Some recombinants capable of producing RPV-type lesions upon intracutaneous (i.c.) infection of rabbits could also produce EMV-specific lesions upon footpad inoculation of mice. The analysis of some recombinants as well as vaccinia virus strains has shown that the ability of the virus to reproduce when injected into the mouse footpad is a necessary, but not a sufficient condition for production of EMV-type lesions. According to restrictase analysis of recombinant DNA, the genome of recombinants mainly consists of RPV DNA sequences with insertions of small EMV DNA fragments.

Key words: ectromelia and rabbitpox viruses; recombinants; specific pathogenicity; restrictase DNA analysis

Introduction

Ectromelia viruses (mousepox and EMV) and rabbitpox viruses (RPV) belong to the genus of orthopoxviruses, whose members are serologically related (Baxby, 1975; Woodrofe, Fenner, 1962), with respect to the number and molecular weight of virion proteins (Arita, Tagaya, 1980; Esposito *et al.*, 1977; Ikuta *et al.*, 1978; Obijeski *et al.*, 1973) and the genome structure (Mackett, Archard, 1979; Wittek, 1982; Holowczak, 1982). Physical mapping by help of restriction endonucleases has shown that the central portion of orthopoxvirus genomes (about 60—65%) was conservative, whereas the end segments were variable (Mackett, Archard, 1979; Holowczak, 1982; Wittek, 1982).

In spite of the obvious similarity of genome structures and structural proteins of these viruses, wide variations of their biologic properties are known. Thus, for example, EMV and RPV differ in most of their biologic markers: temperature-sensitivity, size and character of lesions in tissue culture and in chick embryo chorioallantoic membrane (CAM), as well as in the

spectrum of their pathogenicity for laboratory animals (Fenner, 1958; Ghendon, Chernos, 1964). RPV is highly pathogenic for rabbits — it causes their death after intracerebral, intraperitoneal, epicutaneous and intracutaneous administrations, whereas it is essentially apathogenic for mice upon footpad administration. In contrast, EMV is apathogenic for rabbits by any administration route, but causes specific lesions in mice upon administration into the footpad, resulting in production of a peculiar oedema followed by necrosis and amputation of the limb, as well as a generalized infection leading to death of the animals.

It has been shown that orthopoxviruses can form interspecies recombinants (Woodrofe, Fenner, 1960; Bedson, Dumbell, 1964*a*,*b*; Sitnikov, Ghendon, 1966; Chernos *et al.*, 1983). This lays the basis for the investigation of molecular mechanisms of orthopoxvirus pathogenicity. Previously we had obtained recombinants between EMV and temperature-sensitive mutants of vaccinia virus showing pathogenic properties of both parent viruses, and performed the restrictase analysis of the genome structure of these recombinants (Chernos *et al.*, 1983; Senkevich *et al.*, 1983; Chernos *et al.*, 1985). The purpose of our work has been to produce recombinants between EMV and RPV DNA fragments. EMV does not reproduce or form plaques at 40 °C. This allowed to select at high temperature those recombinants that result from transfection of EMV-infected cells by the cleaved RPV DNA samples. Thus, the prepared recombinants had different sets of properties which provided a basis for detecting the genomic sites responsible for particular biologic properties of the virus, specific pathogenicity included.

Materials and Methods

Viruses and cells. RPV strain Utrecht and EMV strain Mill Hill were taken as initial parents for the preparation of recombinants. The viruses were grown on chick embryo CAMs, purified and concentrated with the use of the method described by Joklik (1962), and kept at −20 °C. Primary chick embryo fibroplast culture (CEF) prepared according to Porterfield and Allison (1960) was used for passages and titration.

Production of recombinants. For the isolation of recombinants, CEF cell monolayers (10^6 cells per flask) were infected with EMV at a multiplicity of infection 0.1−1 PFU per cell. After 1 hr adsorption at 20−22 °C, the cells were washed with medium 199, heated to 37 °C and then restrictase *Sma*I-pretreated RPV DNA preparation (0.2−0.5/μg per 10^6 cells) coprecipitated with calcium phosphate was added (Graham, Van der Eb, 1973). The infected cells were incubated with DNA for 40 min at room temperature. 3 ml of medium 199 were added to the flask without washing and then incubated at 36 °C or 40 °C (depending on the temperature at which the experiment should be carried out). After 3 hr the medium was removed, the cells were washed three times with fresh medium 199, heated to an appropriate temperature (37 °C or 40 °C) and then 7 ml of medium was added; the flasks were incubated for 40−46 hr at 36 °C or 40 °C. Thereafter, the cells were destroyed by 3 freezing-thawing cycles, and the number of plaques under agar at 40 °C was determined. The virus was then picked from the plaques formed at 40 °C, recloned and the genetic markers of the resulting clones were tested. The size and character of the lesions on chick embryo CAMs were assessed using the technique described by Fenner (1958). The pathogenicity of the viruses in rabbits and mice was determined as described by Chernos *et al.* (1983).

Isolation and restrictase analysis of viral DNA. Virus DNA was isolated from infected CEF cells cytoplasm according to the method proposed by Esposito *et al.* (1981). DNA preparations were treated by restrictases *Hind*III, *Xho*I and *Eco*RI and the resulting fragments were electrophoresed in agarose gel as described in our earlier paper (Senkevich *et al.*, 1983).

Table 1. Genetic markers of recombinants between ectromelia virus (EMV) fragmented rabbitpox virus (RPV) DNA

Virus	Genetic markers				
	Reproduction at 40°C	Plaque size[x]	CAM lesions[xx]	Pathogenicity for rabbits[a]	Pathogenicity for mice[b]
RPV	+	L	LH	++	−
EMV	−	S	S	−	+
R 21/1	+	L	LH	++	−
R 21/2	+	M + S	SH	+	−
R 21/2a	+	M	SH	+	−
R 21/2b	+	M	SH	+	−
R 21/2c	+	M	SH	+	+
R 21/2d	+	M	SH	+	−
R 21/2e	+	M	SH	+	+
R 23/1 as well as reclones R 23/1a, R 23/1b, R 23/1c, R 23/1d. R 23/1e	+	M	MH	++	+
R 23/3 as well as reclones R 23/3a R 23/3b, R 23/3c, R 23/3d, R 23/3e	+	L	LH	++	−

[x]S — small up to 1 mm, M — medium: 1—2.4 mm, L — large: 2.5—3 mm
[xx]S — small up to 0.2 mm, L — large: 1—2.5 mm, SH and LH — the same with haemorrhages
[a] Skin lesions without necrosis: +, skin lesions with necrosis: ++
[b] Specific lesions after footpad infection

Isolation and purification of viruses and analysis of virion polypeptide composition. Cultivation of the viruses in the presence of radioactive precursors (^{14}C-amino acids), isolation, purification and gel-electrophoretic technique have been described elsewhere (Chelyapov *et al.*, 1984). The concentrations of acrylamide and bisacrylamide in the running gel were 15% and 0.073%, respectively.

Results

Biological characterization of the recombinants

The materials from the flasks infected with EMV and transfected with split RPV DNA at dilutions 1 and 2 yielded singular plaques under agar overlay at 40 °C on days 5 or 6 of incubation. The materials from control flasks infected with EMV or RPV DNA failed to form plaques in any of these cases. Virus clones isolated from the plaques were tested with respect to several genetic markers. Certain clones appeared to be heterogeneous as to the character of lesions observed in chick embryo CAM. Therefore, they

Table 1 continued

Virus	Genetic markers				
	Reproduction at 40°C	Plaque size[x]	CAM lesions[xx]	Pathogenicity for rabbits[a]	Pathogenicity for mice[b]
R 23/5 as well as reclones R 23/5a, R 23/5b, R 23/5c, R 23/5d, R 23/5e	+	M	LH	++	--
R 23/6 as well as reclones R 23/6a R 23/6b, R 23/6c, R 23/6d	+	L	LH	++	+
R 23/8 as well as reclones R 23/8a, R 23/8b, R 23/8c, R 23/8d, R 23/8e	+	L	LH	++	–
R 23/10 as well as reclones R 23/10a, R 23/10b, R 23/10c, R 23/10d, R 23/10e	+	L	LH	++	–
R 23/13	+	L	LH	+	+
R 23/13a	+	L	LH	++	–
R 23/13b	+	L	LH	++	+
R 23/13c	+	L	LH	++	+
R 23/13d	+	L	LH	++	+
R 23/13e	+	L	LH	++	–
R 23/13f	+	M	LH	+	–

were recloned and tested again. The results of these tests are summarized in Table 1.

As seen from the presented data, EMV and RPV differed markedly in all genetic markers tested. Recombinant R 21/1 was indistinguishable from RPV with respect to all the markers tested. R 21/2 was different from both, EMV and RPV, with respect to plaque sizes on CEF, the size and character of lesions on chick embryo CAMs, the character of lesions caused by intracutaneous infection of rabbits. It is noteworthy that its recloning resulted in the production of 2 subclones which could not only cause lesions after intracutaneous administration to rabbits, but were also responsible for specific lesions after injection into the mice footpad. Out of all orthopoxviruses this property is typical for EMV. R 23/1 and all other subclones isolated from it differed not only from EMV but also from RPV and had a "double" pathogenicity, i.e. causing lesions but no necrosis in rabbits, and causing specific "ectromelian" lesions upon footpad administration in mice. The re-

Table 2. Correlation between virus reproduction in the mouse paw after footpad inoculation and the development of specific »ectromelian« lesions

Virus	Reproduction in the paw (PFU/ml)*	Specific lesions
Ectromelia	5×10^8	+
Rabbitpox	3×10^6	–
Recombinant R 21/2	$> 1 \times 10^7$	–
Recombinant R 23/1	$> 1 \times 10^7$	+
Recombinant R 23/3	5×10^4	–
Recombinant R 23/5	3×10^4	–
Recombinant R 23/6	$> 1 \times 10^7$	+
Recombinant R 23/8	2×10^6	–
Recombinant R 23/13	$> 1 \times 10^7$	+
Recombinant R 23/10	$> 1 \times 10^7$	–
Vaccinia virus strain LIVP	1×10^3	–
Vaccinia virus strain Tomsk	7×10^6	–
Vaccinia virus strain Minsk	1×10^3	–
Vaccinia virus strain WR	1×10^9	–
Vaccinia virus strain MM	8×10^3	–

* Groups of 10 animals were injected into the footpad with 1×10^4 PFU of the virus tested. On day 6 p.i. animals in each group were sacrificed, the paws were removed to prepare 10% suspensions. The rest of the animals was observed for 14 days.

combinant R 23/3 and all subclones isolated from it were essentially identical to RPV and to R 23/5; its subclones differed only in the plaque size on CEF.

The comparison of R 23/6 recombinant and its subclones with recombinant R 23/8 and its subclones indicates that they are similar with respect to all genetic markers and indistinguishable from RPV; however, recombinant R 23/6 and its subclones had a marked "double" pathogenicity, the latter being even more marked than with the initial EMV during infection of mice into footpad. Some subclones of recombinant R 23/13 were pathogenic for mice upon footpad inoculation and some were apathogenic by this administration route, although they were indistinguishable in all other markers tested.

It follows from the presented data that recombination of EMV with RPV DNA fragments yielded recombinants with different genetic patterns. Most important was that recombinants pathogenic both for rabbits by intracutaneous infection and for mice by footpad injection were obtained. The analysis of a large number of recombinants has shown that the ability to cause specific lesions in mice was transferred independently and was not correlated with the transfer of any other genetic marker tested. In the recombinants with "double" pathogenicity after the first cloning, this property as well as other their genetic markers were conserved throughout several passages in tissue culture and also after passages in mice. Independent transfer of the ability to cause EMV-specific lesions seemed to be an adequate pre-

requisite for the attempts to identify a particular area of EMV genome responsible for this property and also to use this model for the investigation of molecular mechanisms of pathogenicity.

First of all it was necessary to find out whether the ability of EMV to cause specific lesions in mice after footpad inoculation was related to any other factor or it was soly predetermined by virus reproduction itself. With this aim we tested the ability of certain orthopoxviruses to reproduce in the footpad tissues after skin inoculation. The results of these experiments are summarized in Table 2. It can be seen that EMV, as well as the recombinants R 23/1, R 23/6, and R 23/13 which acquired the ability of causing specific "ectromelian" lesions reproduced in the mouse limb tissues to high titres. Meanwhile, other recombinants (R 21/2, R 23/10) that also could reproduce in the mouse limb tissues to high titres failed to cause any lesions.

This conclusion was further strengthened by the study of the ability of some vaccinia virus strains to reproduce after intracutaneous infection in mice. Strain WR reproduced more actively than EMV did after administration by this route. Although the infection with WR strain was sometimes associated with swelling of the limb, this sign disappeared on observation days 10—12 and never turned into necrosis so that the character of changes was quite different from those caused by EMV or its pathogenic recombinants. Thus, the reproduction in the limb tissues to high titres was a necessary but not a sufficient condition for the production of specific "ectromelian" lesions. There are a few viruses that can actively reproduce in the limb tissues after intracutaneous infection and yet they do not cause such lesions.

Analysis of the genome structure of recombinants

For the analysis of genome structure of the recombinants combined electrophoresis of *Hind*III, *Xho*I and *Eco*RI fragments of parent and recombinant DNAs has been carried out. Although fragmented RPV DNA has been used for the preparation of recombinants, it follows from the given data (Fig. 1) that most fragments of recombinant DNAs have the same electrophoretic mobility as the RPV DNA fragments, and distinct of the EMV DNA fragments. Consequently, RPV genetic information is predominant in the recombinant genome. However, the DNA of all the recombinants tested appeared to be different from RPV DNA, as evident from the analysis with each of the 3 restrictases. These dissimilarities were most marked in the righthand portion of the genome. Thus, for example, in the DNA of all the recombinants presented on Figs 1—4, the *Hind*III-A- and *Hind*III-C-fragments of RPV DNA were missing, i.e. the righthand portion of the genome has been exchanged. This could be expected, for parent RPV DNA was treated with restrictase *Sma*I having only 1 cleavage site in this DNA at a distance of $\sim$ 18 MD from the righthand end (Mackett, Archard, 1979). In R 23/13b and R 23/10a DNAs, *Hind*III fragment was detected having the same electrophoretic mobility as *Hind*III-1 EMV fragment located near the righthand end of the genome (Mackett, Archard, 1979). However, the data presented are insufficient for the assessment of the size of EMV DNA insert-

ion in this area of the genome, as fragments whose electrophoretic mobility were different from both parents had been detected. Moreover, some recombinants seem to contain additional EMV DNA insertions in other genome areas. For example, R 23/6 genome appeared to have an EMV DNA insertion in the central portion of the genome. It should be also noticed that according to restriction analysis, all the recombinants were not only different from their parents, but also from each other. In some cases even subclones isolated from the same clone appeared to be dissimilar (Figs. 1—3).

In the electrophoregrams shown in Fig. 1 the fragments present in lower molar quantities than the majority of the fragments deserve special attention. These submolar fragments do not result from incomplete hydrolysis by restrictases. Relative quantity of submolar fragments varies in different subclones of the same clone (R 23/13a-b, Figs. 1—3) from 0 to 1 M. The presence of submolar fragments in the genome of some of the recombinants may be indicative of structural rearrangements in the genomes of these viruses. A similar situation has been described for some cowpox virus mutants (Archard *et al.*, 1984). The recombinants between EMV and vaccinia virus containing an EMV DNA insertion in the central portion of the genome described in our previous paper usually were homogeneous after single cloning (Chernos *et al.*, 1985).

Electrophoretic analysis of recombinant structural proteins was carried out in the gels containing 15% of acrylamide and 0.073% of bisacrylamide. In these gels high-molecular polypeptides cover longer distances than in the standard (10—12.5%) polyacrylamide gels (Essani, Dales, 1979; Oie, Ichihashi, 1981), and other polypeptides can still be clearly identified. The comparison of electrophoregrams of parent RPV and EMV proteins (Fig. 2) shows that although they are strinkingly similar, they still have a few characteristic marker proteins. EMV has a high-molecular polypeptide p160, and two polypeptides in the vicinity of 130K are located closer to each other, as compared to the analogous proteins in RPV. RPV had a structural protein p92 that was absent in EMV; EMV had a protein p82, and RPV — p80. RPV polypeptide pattern was characterised by protein p64, that was absent in EMV, as well as some other polypeptides.

In most cases, the polypeptide pattern of recombinants is more similar to that of RPV, i.e. they carry more marker RPV proteins. However, there are a few peculiarities. Thus, for example, proteins near 130K in the recombinant R 23/1a are analogous to those of EMV. This recombinant also has a protein 82K that is characteristic of EMV. Recombinant R 23/6a has the uncommon polypeptide pattern that is a mixture of EMV and RPV marker proteins. It has the same proteins as RPV near 130K, however, it does not have protein 92K that is characteristic of RPV, but has the EMV protein 82K. Protein pattern of recombinant R 23/6a in the area 65—68K is different from both of the parents. A redistribution of the intensities of protein bands was observed. A similar picture has been observed in the 20K area in R 23/13b. In the rest of recombinants the protein patterns were similar to that of RPV showing insignificant variations within range of methodologic limits.

Discussion

Preparation of orthopoxvirus interspecies recombinants and the attempts to correlate their biologic properties with genome structure is one of the most important approaches to the investigation of the functions of particular areas of the genome. For good progress of these investigations methods for production of necessary recombinants are needed. We have previously obtained a few recombinants between vaccinia virus ts-mutants and EMV and have shown that some of them have acquired "double" pathogenicity, i.e. they had both an ability to cause lesions in rabbits on intracutaneous infection characteristic of vaccinia virus and an EMV-specific ability to cause lesions in mice after infection into the footpad (Chernos *et al.*, 1983; Senkevich *et al.*, 1983; Chernos *et al.*, 1985). However, the method of selective isolation of recombinants based on the inability of both parent strains to form plaques in tissue culture at 40 °C yielded as little as 4 recombinants with "double" pathogenicity out of more than 100 recombinants tested.

We have shown in this paper that the transfection of EMV-infected cell culture with RPV nucleic acid treated with restrictase *Sma*I results in selective production of recombinants with a wide variety of properties (Table 1). It is especially important that most part (about 30%) of thus produced recombinants acquires EMV-specific pathogenicity, and at the same time retains many RPV genetic features, including pathogenicity by intracutaneous infection in rabbits. Of importance is also whether the ability of EMV to cause specific lesions in mice after footpad administration was related to the ability to replicate by this administration route only, or to another factor (or factors) involved. It has been shown in this paper that some orthopoxviruses can reproduce in mouse footpad at least as intensively as EMV or its pathogenic recombinants, but they do not cause specific lesions (Table 2). In some cases, recombinants apathogenic for mice (for example, R 23/10) replicate after injection into the footpad as intensively as the pathogenic ones, i.e. R 23/1, R 23/6, or R 23/13.

Most recombinants were similar to RPV with respect to virion protein pattern, although in some of them (R 23/1a, R/23/6a) the marker proteins characteristic of EMV were detected (Fig. 1). These data are in good agreement with the results of recombinant DNA analysis. Restrictase analysis of the recombinant DNAs has shown that although RPV genetic material is predominant in their genome, they are all different from both, RPV and from each other, the differences being evident from the analyses with each of the three restrictases. It cannot be entirely ruled out that some of the clones tested resulted not from recombination, but from ligation of the two fragments of RPV DNA. DNA of such isolates may differ from RPV DNA because of genomic rearrangements (deletions and translocations) in the area adjacent to *Sma*I restrictase cleavage site. However, restrictase analysis of the DNAs of most isolates detected fragments with an electrophoretic mobility equal to that of EMV DNA fragments, which indicates that they are true recombinants. These data are in good accord with the fact that a large number (about 30%) of recombinants acquired EMV-specific pathogenicity. In some of recom-

binants pathogenic for mice EMV DNA insertion has been detected as expected, in the righthand portion of the genome near to the parent RPV DNA cleavage site. These findings as well as frequent occurrence of recombinants pathogenic for mice provided by this method indicates a probable location of EMV pathogenicity determinant at the righthand end of the genome. However, the recombinant R 23/6 highly pathogenic for mice and all its reclones have an additional EMV DNA insertion in the central portion of the genome. This insertion is located in the same DNA region, as in the earlier described recombinants between EMV and vaccinia virus pathogenic for mice (Chernos *et al.*, 1985). The mapping of EMV pathogenicity determinant on the basis of our data is also hindered by the complex genome structure of the recombinants. As it has been mentioned above, the restrictase cleavage pattern of recombinant DNAs indicates that in some cases recombination was associated with not only EMV DNA integration into colinear RPV genome regions, but also with genome rearrangements affecting its righthand portion. It is well known that the regions in the vicinity of orthopoxvirus DNA ends contain genes irrelevant for the reproduction in the tissue culture, and are often subject to deletions and translocations (Wittek, 1982). Thus, the present paper has described a method which allows to obtain high frequency recombinants with EMV-specific pathogenicity, with predominant RPV genetic material in their genome. Precise localization of EMV DNA insertions in the genome of such recombinants will make possible the mapping of EMV pathogenicity determinant.

References

Archard, L. C., Mackett, M., Barnes, D. E. and Dumbell, K. R. (1984): The genome structure of cowpox virus white pock mutants. *J. gen. Virol.* **65**, 875–886.

Arita, M. and Tagaya, I. (1980): Virion polypeptides of poxviruses. *Arch. Virol.* **63**, 209–225.

Baxby, D. (1975): Identification and interrelationship of the variola/vaccinia subgroup of poxviruses. *Prog. med. Virol.* **19**, 215–246.

Bedson, H. S. and Dumbell, K. R. (1964*a*): Hybrid derived from the viruses of alastrim and rabbitpox. *J. Hyg. (Camb.)* **62**, 141–146.

Bedson, H. S. and Dumbell, K. R. (1964*b*): Hybrids derived from the viruses of variola major and cowpox. *J. Hyg. (Camb.)* **62**, 147–158.

Chelyapov, N. V., Antonova, T. P., Tarasov, L. F. and Chernos, V. I. (1984): Electrophoretic analysis of virion proteins of recombinants between ectromelia virus and temperature-sensitive mutant of vaccinia virus (in Russian). *Mol. Genet. Mikrobiol. Virusol.* **12**, 29–34.

Chernos, V. I., Antonova, T. P., Mitina, I. V. and Senkevich, T. G. (1983): Study of genetic markers of recombinants between ectromelia virus and DNA$^-$ temperature-sensitive mutants of vaccinia virus (in Russian). *Mol. Genet. Mikrobiol. Virusol.* **10**, 24–28.

Chernos, V. I., Antonova, T. P. and Senkevich, T. G. (1985): Recombinants between vaccinia and ectromelia viruses bearing specific pathogenicity markers of both parents. *J. gen. Virol.* **66**, 621–625.

Esposito, J., Condit, R. and Obijeski, J. (1981): The preparation of orthopoxvirus DNA. *J. virol. Meth.* **2**, 175–179.

Esposito, J. L., Obijeski, J. F. and Nakano, J. H. (1977): The virion and soluble antigen proteins of variola, monkeypox and vaccinia viruses. *J. med. Virol.* **1**, 95–110.

Essani, K. and Dales, S. (1979): Biogenesis of vaccinia: evidence for more than 100 polypeptides in the virion. *Virology* **113**, 263–276.

Fenner, F. (1958): The biologic characters of several strains of vaccinia, cowpox and rabbitpox viruses. *Virology* **5**, 502—529.
Ghendon, Yu. Z. and Chernos, V. I. (1964): Comparative study of genetic markers of some poxviruses strains. *Acta virol.* **8**, 359—342.
Graham, F. L. and Van der Eb, A. L. (1973): A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456—457.
Holowczak, J. A. (1982): Poxvirus DNA. *Curr. Top. Microbiol Immunol.* **97**, 27—79.
Ikuta, K., Miyamoto, H. and Kato, S. (1978): Studies on the polypeptides of poxvirus I. Comparison of structural polypeptides in vaccinia, cowpox and shope fibroma viruses. *Biken J.* **21**, 51—61.
Joklik, W. K. (1962): The purification of four strains of poxviruses. *Virology* **18**, 9—18.
Mackett, M. and Archard, L. C. (1979): Conservation and variation in orthopoxvirus genome structure. *J. gen. Virol.* **45**, 683—701.
Obijeski, J. F., Palmer, E. L., Gafford, L. and Randall, C. C. (1973): Polyacrylamide gel electrophoresis of fowlpox and vaccinia proteins. *Virology* **51**, 512—516.
Oie, M. and Ichihashi, Y. (1981): Characterization of vaccinia polypeptides. *Virology* **113**, 263 — 276.
Porterfield, I. and Allison, A. P. (1960): Studies with poxvirus by an improved plaque technique. *Virology* **10**, 233—244.
Senkevich, T. G., Antonova, T. P. and Chernos, V. I. (1983): Analysis of the genome structure of recombinants between ectromelia virus and DNA$^-$ temperature-sensitive mutants of vaccinia virus (in Russian). *Mol. Genet. Mikrobiol. Virusol.* **10**, 24—28.
Sitnikov, B. S. and Ghendon, Yu. Z. (1966): Preparation of poxviruses recombinants under the conditions of non-genetic reactivation of rct$^+$-strain by rct$^-$ strain (in Russian). In: *Genetika mikroorganizmov*, 169—174, Moscow.
Wittek, R. (1982): Organization and expression of the poxvirus genome. *Experientia* **38**, 285—297.
Woodrofe, G. and Fenner, F. (1960): Genetic studies with mammalian poxviruses IV Hybridization between several different poxviruses. *Virology* **12**, 272—282.
Woodrofe, G. and Fenner, F. (1962): Serological relationship within the poxvirus group: an antigen common to all members of the group. *Virology* **16**, 334—341.

Legends to the Figures (Plates XXXIX—XLII):

Figs 1—3. Agarose gel electrophoresis of DNA restriction fragments of recombinants and parents obtained by restrictase hydrolysis with *Eco*RI (1), *Hind*III (2), *Xho*I (3). The letters in Fig. 2 (A, B, C, I) indicate rabbitpox virus DNA fragments missing in some recombinants (in the left) and ectromelia virus DNA fragments present in recombinants (in the right).

Fig. 4. Electrophoregrams of the polypeptides of ectromelia virus, rabbitpox virus and their recombinants in the gel with 15% T and 0.47% C.
The bars to the left of the columns corresponding to polypeptides of the viruses tested indicate the "marker" polypeptides. The figures on either side of the electrophoregrams shown the molecular mass of the polypeptides.