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Deletion of a 9,000-Base-Pair Segment of the Vaccinia Virus Genome That Encodes Nonessential Polypeptides

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Deletions contained within the genomes of unstable and stable variants of vaccinia virus (strain WR) were analyzed. Restriction endonuclease mapping and hybridization to specific ^{32}P-labeled DNA probes indicated that more than 6×10^6 daltons of DNA were deleted from the variants. In each case, the deletion occurred on the left side of the genome and started very close to the junction of the inverted terminal repetition and unique sequence. Both variants also contained a new *Sst*I site on the right side of the genome. Hybridization selection and cell-free translation experiments indicated that these variants lost the ability to synthesize at least eight early mRNA's mapping within the deleted region. Although the deleted DNA was not essential for replication of the WR strain of vaccinia virus under laboratory conditions of infection, it presumably has a defined role under other circumstances. This conclusion was based on the conservation within the Elstree strain of vaccinia, the Utrecht strain of rabbitpox, and the Brighton strain of cowpox virus of sequences homologous to the deleted DNA. Moreover, mRNA's that hybridized to the deleted vaccinia virus DNA segment and encoded similar size polypeptides were made in cells infected with rabbitpox and cowpox viruses.

Poxviruses are large DNA viruses that replicate within the cytoplasm of infected cells (13). Vaccinia virus, the most intensively studied member of this group, has a linear, double-stranded DNA genome of approximately 120×10^6 daltons (180,000 base pairs [bp]) with covalently cross-linked ends that prevent the DNA strands from separating upon denaturation (7, 8). Complementary sequences present at the two ends of the genome form a 10,000-bp inverted terminal repetition (6, 26). Within this long repetition, there are two sets of about 15 tandemly repeating 70-bp units (28) as well as a region encoding early mRNA's (3, 23, 24).

Restriction endonuclease analysis of DNA from serially passaged vaccinia virus stocks revealed heterogeneity in the lengths of terminal fragments that was eliminated by plaque purification (11, 29). Analysis of individual plaque isolates indicated that 20% of them contained deletions of up to 250 bp within the inverted terminal repetition (12). It is likely that this microheterogeneity results primarily from variations in the number of 70-bp repeating units (14). However, deletions beyond the inverted terminal repetition also contribute to the observed heterogeneity of vaccinia virus (17). Even larger deletions or rearrangements near the ends of the genome have been found in rabbitpox (15, 16), cowpox (1), and monkeypox (5) virus isolates.

The presence of a large deletion was noted during the analysis of an unstable variant of vaccinia virus (14). This variant appears to undergo frequent recombinational events leading to the reiteration of entire sets of 70-bp tandem repeats and adjacent DNA. The deletion, however, appeared to have no direct relationship to the phenomenon of instability since stable revertants still had the deletion and, moreover, some independently isolated unstable variants had apparently complete genomes. Nevertheless, the deletion was of considerable interest to us because it seemed to be in an actively transcribed part of the genome. Indeed, we have now mapped this 9,000-bp deletion and demonstrated that at least eight early mRNA's encoded within this region are not synthesized in cells infected with the variants. Evidently these mRNA's are not necessary for virus replication under laboratory conditions. Nevertheless, homologous DNA sequences and corresponding mRNA's are present in rabbitpox and cowpox, as well as another vaccinia strain. Conservation of this DNA segment implies that the mRNA's serve a useful function under other conditions of infection. One possible function is discussed.

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MATERIALS AND METHODS

Virus and cell culture. Standard and variant viruses from the serially passaged stock of vaccinia (strain WR) were isolated as plaques on BSC-1 monolayers. Variants 6/1 and 6/2 were obtained by replaquing isolate 6 (14). Similarly, vaccinia virus (strain Elstree), rabbitpox (strain Utrecht), and cowpox (strain Brighton) were plaque purified two times in succession. All poxviruses were purified from infected HeLa cells by a modification of the procedure of Joklik (9).

Restriction endonuclease analysis. DNA was isolated from purified virus (6) and cleaved with restriction endonucleases as recommended by the manufacturers. DNA fragments were resolved by electrophoresis on 0.6, 0.8, or 1% agarose gels, stained with ethidium bromide, and photographed by UV transillumination.

Hybridization of DNA. DNA fragments were transferred from agarose gels to nitrocellulose membranes by a modification (22) of the Southern (20) procedure. Nick-translated (19) recombinant DNA or DNA fragments purified from agarose gels by binding to glass powder (21) were hybridized to immobilized DNA as described (14). Autoradiographs or fluorographs were made by placing the nitrocellulose sheets in contact with X-ray film for 1 to 4 days.

Restriction endonuclease maps. *Hin*dIII and *Sst*I maps of the Elstree strain of vaccinia virus were constructed originally by Wittek and co-workers (27). Mackett and Archard (10; personal communication) derived *Hin*dIII, *Xho*I, and *Sst*I maps for additional vaccinia virus strains including WR. A similar *Hin*dIII map of vaccinia virus strain WR used in our laboratory was made by F. DeFilippes (personal communication). The order of the small *Hin*dIII N and M fragments was reported by Belle Isle et al. (H. Belle Isle, S. Venkatesan, and B. Moss, Virology, in press).

To confirm the *Xho*I and *Sst*I restriction endonuclease maps for our WR isolate, cloned *Hin*dIII fragments D, E, F, G, H, I, J, K, L, M, N, and O (Belle Isle et al., in press), agarose gel-purified *Hin*dIII A, B, and C fragments, and closed *Eco*RI fragments of *Hin*dIII C (23, 25) were labeled with ^{32}P and individually hybridized to electrophoretically separated *Xho*I and *Sst*I digests of vaccinia DNA immobilized on nitrocellulose. The results were consistent with the maps of Mackett and Archard (10), with the exception that, in our virus strain, the *Xho*I J fragment was on the right rather than the left side of the genome (see Fig. 1). *Xho*I digests of purified *Hin*dIII B and C fragments were analyzed by agarose gel electrophoresis to prove this point. A similar location of the *Xho*I J fragment was reported by Panicalli et al. (17) on a strain of virus originally obtained from our laboratory.

Hybridization selection of RNA and cell-free translation. RNA was selected by hybridization to DNA immobilized on nitrocellulose filters in 80% formamide–0.4 M NaCl–0.04 M Na piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (pH 6.4)–0.001 M EDTA–0.1% sodium dodecyl sulfate at 37°C for 18 h. At the end of this time, the filters were stringently washed (3). The hybridized RNA was eluted in 0.2 ml of water at 100°C for 2 min and alcohol precipitated with 20 μg of calf liver tRNA. RNA was translated by the message-dependent reticulocyte lysate in 5- or 10-μl reactions containing 2 μCi of [^{35}S]methionine per μl (2). Samples of the in vitro translations were analyzed by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels (2). Radioactive proteins were detected by fluorography using preexposed Kodak XR film, after impregnation of the gel with 2,5-diphenyloxazole or with Enhance, a product of New England Nuclear Corp.

Materials. Restriction endonucleases were purchased from New England Biolabs or Bethesda Research Laboratories. Radioisotopes were manufactured by Amersham Corp.

RESULTS

Size and location of the deletion. Previously, we noted that plaque isolate 6, obtained from a serially passaged stock of vaccinia virus (strain WR), gave rise to two types of progeny on further plaque purifications (14). Stable ones such as 6/2 had the usual sequence arrangement near the end of the inverted terminal repetition: a unique segment of about 450 bp flanked on both sides by tandem 70-bp repeats. DNA isolated from unstable progeny such as 6/1 consisted of a family of molecules of varying length. The variation was due to tandem reiterations of a 1.1×10^6-dalton unit consisting of both the 450-bp sequence and a set of about 15 tandem 70-bp repeats. In addition, both stable and unstable variants contained a large deletion that appeared to be unrelated to the phenomenon considered above.

The size and location of the deletion was determined by restriction endonuclease analysis. Agarose gel electrophoresis of *Hin*dIII digests indicated that the left terminal C fragment was missing from 6/1 and 6/2 (Fig. 1). Gels loaded with more DNA clearly showed that the *Hin*dIII N fragment also was missing. However, a new fragment just below *Hin*dIII F was present in 6/2 (Fig. 1). Although that additional band was not seen in 6/1, a faint submolar band below E was detected. To more fully understand the nature of these changes, the *Hin*dIII fragments were transferred from an agarose gel to a nitrocellulose membrane, and duplicate strips were hybridized to individual ^{32}P-labeled DNA fragments. Results obtained using the 2.2×10^6-dalton *Hpa*II end fragment, which is contained within the inverted terminal repetition and the *Hin*dIII N fragment, are shown in Fig. 2. As expected, the *Hpa*II fragment hybridized to the two end fragments, *Hin*dIII B and C, of our standard virus. It also hybridized to the *Hin*dIII B fragment of 6/2 as well as to a smaller one of 8.5×10^6 daltons. The *Hin*dIII N fragment of standard virus, which is missing from the variants, also hybridized to the new 8.5×10^6-dalton *Hin*dIII fragment of 6/2. Accordingly, only a

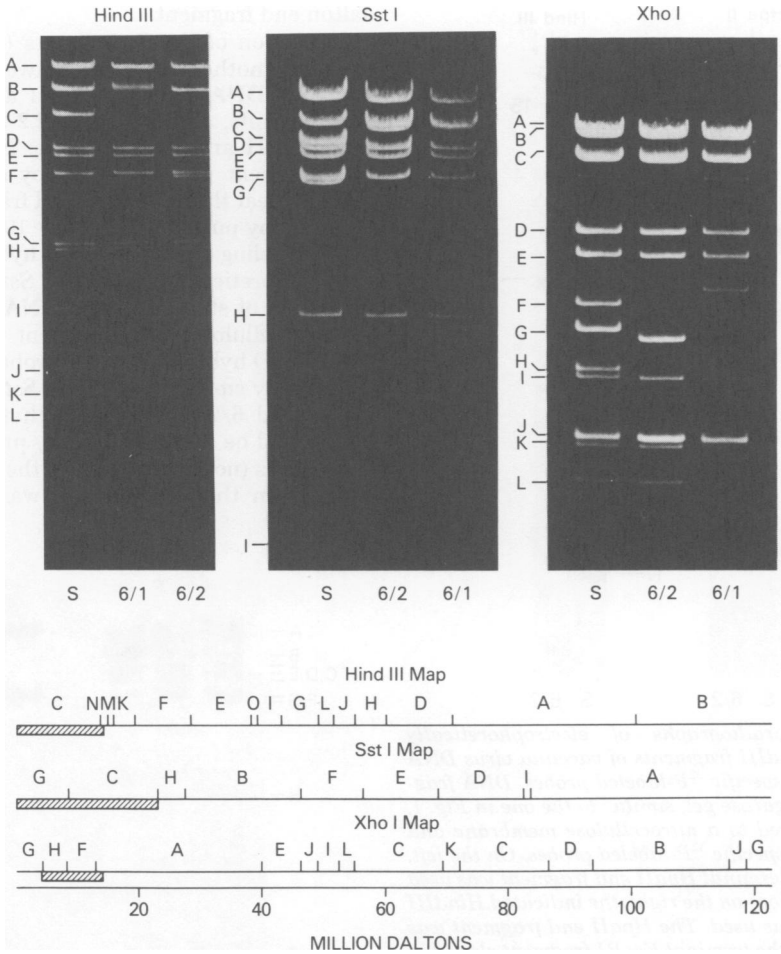

FIG. 1. *Stained agarose gels containing separated HindIII, SstI, and XhoI DNA fragments of vaccinia virus. Approximately 1 μg of vaccinia virus DNA was digested with the indicated restriction endonuclease and analyzed by electrophoresis on 0.6% agarose gels. Gels were stained with ethidium bromide, transilluminated with UV light, and photographed. S, Standard laboratory plaque purified isolate. 6/1 and 6/2, Plaque isolates containing deletion. HindIII fragments N, M, and O were too faint to be reproduced photographically. Examination of this gel, as well as gels loaded with more HindIII-digested DNA, revealed that HindIII N was missing from 6/1 and 6/2. HindIII, SstI, and XhoI maps shown in the lower part of the figure are described in the text. Fragments missing from 6/1 and 6/2 are indicated.*

portion of the *Hin*dIII N fragment was deleted. Therefore, the deletion must begin in the *Hin*dIII C fragment and stop in the *Hin*dIII N fragment. The new 8.5×10^6-dalton fragment was formed by fusion of the terminal part of *Hin*dIII C to the remaining part of *Hin*dIII N. Since the *Hin*dIII C and N fragments together are about 14.5×10^6 daltons and the new end fragment is 8.5×10^6 daltons, the deletion must be about 6×10^6 daltons.

Similar results were also obtained by hybridizing the same two labeled probes to *Hin*dIII digests of 6/1 DNA, except that the appearance of the autoradiograph was complicated by multiple submolar bands caused by the reiterations discussed above. An autoradiograph displaying the ladder-like series of *Hin*dIII fragments that hybridized to the *Hpa*II end fragment has been published previously (14). The lowest band of the set comigrated with *Hin*dIII F and is therefore slightly larger than the corresponding fragment of 6/2, apparently because of additional 70-bp repeats (14). The next larger band in the ladder corresponds to the faint band below E seen in Fig. 1. To clearly see the ladder pattern of submolar bands in stained gels, it is necessary to overload with DNA and to prolong the electrophoresis time.

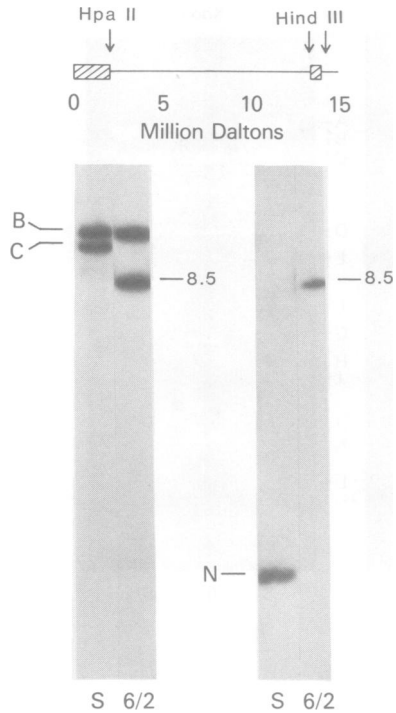

FIG. 2. *Autoradiographs of electrophoretically separated HindIII fragments of vaccinia virus DNA hybridized to specific ^{32}P-labeled probes. DNA fragments in an agarose gel, similar to the one in Fig. 1, were transferred to a nitrocellulose membrane and hybridized to specific ^{32}P-labeled probes. On the left, the indicated terminal HpaII end fragment was used for hybridization; on the right, the indicated HindIII N fragment was used. The HpaII end fragment was isolated from the terminal EcoRI fragment cloned in phage λ (23); the HindIII N fragment was cloned in pBR322 (Belle Isle et al., in press).*

Analysis of *Sst*I digests of 6/1 and 6/2 DNA revealed that fragments C and G, which total about 24.2 × 10^6 daltons, were missing. However, a new band of about 18 × 10^6 daltons just below B was resolved in 6/2. To identify the terminal *Sst*I fragments, electrophoretically separated restriction endonuclease digests were transferred to a nitrocellulose membrane and hybridized to total DNA and to a previously cloned 6 × 10^6-dalton terminal *Eco*RI fragment (23) labeled with ^{32}P (Fig. 3). The latter hybridized to *Sst*I end fragments A and G of standard virus. With DNA from 6/1 and 6/2, the probe hybridized to the *Sst*I A fragment as well as to a new end fragment of 18 × 10^6 daltons. The results are consistent with a 6 × 10^6-dalton deletion leading to the fusion of the terminal portion of the *Sst*I G fragment to the remaining portion of the *Sst*I C fragment, thereby generating a new 18 × 10^6-dalton end fragment.

Inspection of the *Sst*I digests (Fig. 1 and 3) revealed another difference between our standard virus DNA and that of 6/1 and 6/2. With both variants, a new band of 2.2 × 10^6 daltons (between H and I) was visible. Evidence that this fragment was generated by an additional *Sst*I site near the left end of *Sst*I fragment A was obtained by purifying the 2.2 × 10^6-dalton fragment, labeling it with ^{32}P, and hybridizing it to electrophoretically separated *Sst*I and *Xho*I fragments of standard virus DNA immobilized on nitrocellulose. *Sst*I fragment A and *Xho*I fragment D hybridized to the probe. In addition, the slightly smaller size of the *Sst*I A fragment of 6/1 and 6/2 compared to that of standard virus could be demonstrated by prolonged electrophoresis (not shown). Thus, the new *Sst*I site is far from the deletion and was presumably

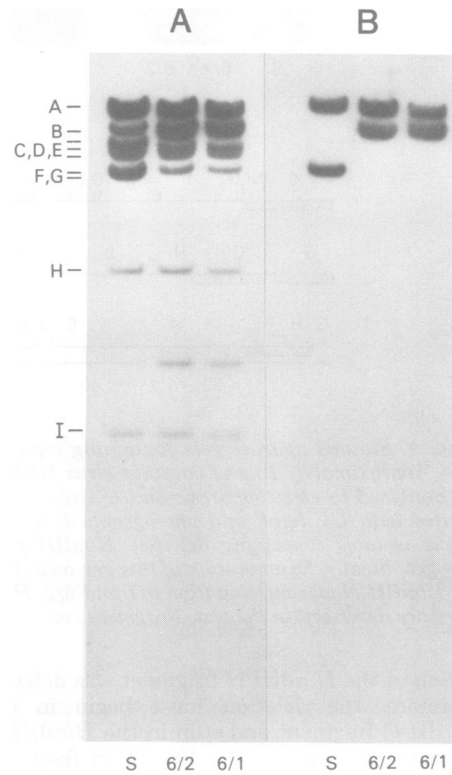

FIG. 3. *Autoradiographs of electrophoretically separated SstI fragments of vaccinia virus DNA hybridized to ^{32}P-labeled probes. DNA fragments in an agarose gel similar to the one in Fig. 1 were transferred to a nitrocellulose membrane and hybridized to total vaccinia virus DNA (A) and the terminal 6 × 10^6-dalton EcoRI end fragment (cloned in phage λ) (B) labeled with ^{32}P by nick translation. S, 6/1, and 6/2 refer to standard and variant vaccinia virus plaque isolates.*

formed by an unrelated genetic event.

Analysis of the *Xho*I digests in Fig. 1 indicated that fragments F and H were missing from 6/1 and 6/2 DNA. The terminal G fragments from both ends of the 6/2 genome were still present in double molar amount, although they were slightly smaller than the standard G fragment because of an apparently unrelated difference in the number of 70-bp repeats (14). Because of the reiterations in 6/1, the G fragment was present in submolar amount, and an additional higher-molecular-weight band below E was visible. When electrophoretically separated *Xho*I fragments of 6/2 DNA were annealed to the ^{32}P-labeled *Hpa*II end fragment, only the one similar in size to *Xho*I G hybridized (14). By contrast, when the *Xho*I fragments of 6/1 were annealed to this probe, a ladder-like array of fragments with 1.1 × 10^6-dalton increments hybridized. The results with 6/1 and 6/2 were consistent, with a deletion leading to the fusion of the distal portion of the H fragment with the proximal part of the A fragment.

In summary, analysis of *Hin*dIII, *Sst*I, and *Xho*I digests indicated that variants 6/1 and 6/2 contain a deletion of about 6 × 10^6 daltons that begins in the *Xho*I H fragment and ends in the *Hin*dIII N fragment.

Further localization of the deletion. To more precisely define the left end of the deletion, ^{32}P-labeled DNA from the 6/1 and 6/2 variants was hybridized to the four electrophoretically separated fragments obtained by *Eco*RI digestion of *Hin*dIII C of standard virus. In control experiments, ^{32}P-labeled standard virus DNA hybridized to all four *Eco*RI fragments (Fig. 4).

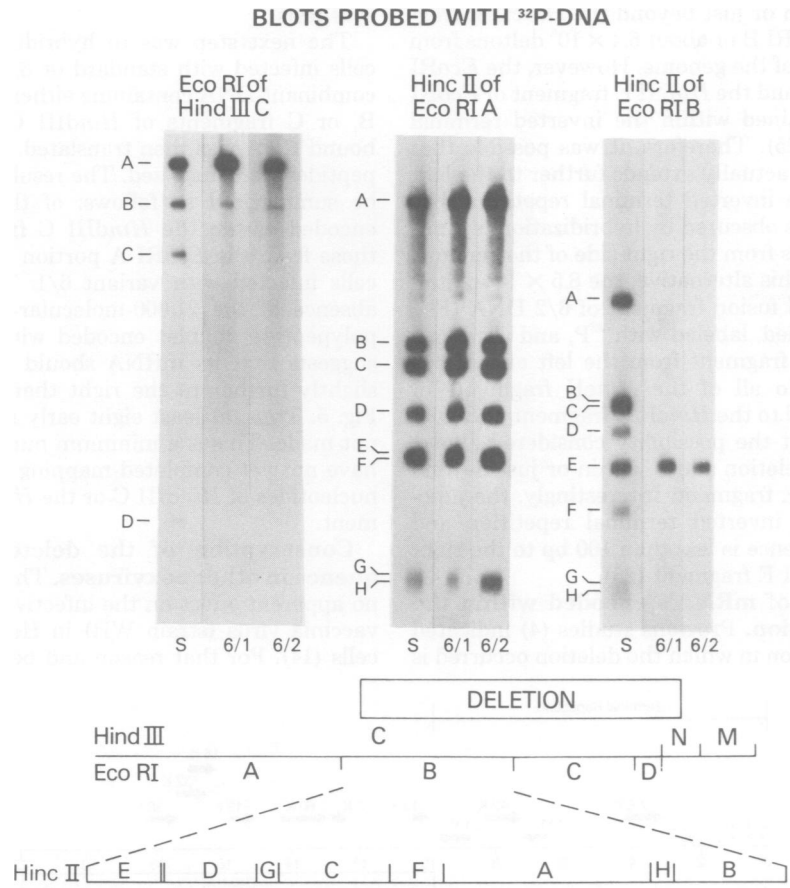

FIG. 4. *Autoradiograph of electrophoretically separated EcoRI and HincII DNA fragments hybridized to* ^{32}*P-labeled vaccinia virus DNA. An EcoRI digest of purified HindIII C fragment and HincII digests of λ recombinants containing the EcoRI A or EcoRI B fragments of HindIII C were resolved by 1% agarose gel electrophoresis and transferred to nitrocellulose membranes. Total DNA from standard (S) and variant (6/1 and 6/2) vaccinia virus was nick translated and used for hybridization. The map at the bottom shows the HindIII sites near the left end of the genome (see Fig. 1), the EcoRI sites within the HindIII C fragment, and the HincII sites within the EcoRI B fragment of HindIII C. The position and extent of the deletion are shown.*

However, DNA from 6/1 and 6/2 hybridized predominantly to fragment A, slightly to B, and not at all to C or D.

Further refinements in mapping were made using the *Eco*RI A and B fragments of *Hin*dIII C which were cloned in phage λ (23, 25). Recombinant DNAs were digested with restriction endonuclease *Hin*cII, and the electrophoretically separated fragments were transferred to nitrocellulose membranes. Parallel strips containing the immobilized *Hin*cII fragments were hybridized to ^{32}P-labeled DNA from standard virus, 6/1, and 6/2. All three probes hybridized to each of the *Hin*cII fragments of *Eco*RI A (Fig. 4). However, the 6/1 and 6/2 probes only hybridized to *Hin*cII E fragment of *Eco*RI B. As illustrated in the bottom of Fig. 4, fragment E of *Eco*RI B is contiguous with the *Eco*RI A fragment. These experiments suggested that the deletion begins either within or just beyond the *Hin*cII E fragment of *Eco*RI B or about 6.4×10^6 daltons from the left end of the genome. However, the *Eco*RI A fragment and the *Hin*cII E fragment of *Eco*RI B are contained within the inverted terminal repetition (25). Therefore, it was possible that the deletion actually extends further to the left, i.e., into the inverted terminal repetition, but that this was obscured by hybridization of similar sequences from the right side of the genome. To explore this alternative, the 8.5×10^6-dalton *Hin*dIII C-N fusion fragment of 6/2 DNA (Fig. 1) was purified, labeled with ^{32}P, and used as a probe. This fragment from the left end of 6/2 hybridized to all of the *Hin*cII fragments of *Eco*RI A and to the *Hin*cII E fragment of *Eco*RI B, ruling out the possibility considered above. Thus, the deletion starts within or just beyond the *Hin*cII E fragment. Interestingly, the junction of the inverted terminal repetition and unique sequence is less than 100 bp to the right of the *Hin*cII E fragment (25).

Absence of mRNA's encoded within the deleted region. Previous studies (4) indicated that the region in which the deletion occurred is actively transcribed. In Fig. 5 the deletion has been aligned with a map of early translational products encoded within the left 20,600 bp of the genome. The following experiments were designed to determine whether mRNA's assigned to this region of the genome are made in cells infected with the deletion mutants.

HeLa cells were treated with cycloheximide to prevent protein synthesis and infected with standard or 6/1 virus. After 4 h, total cytoplasmic RNA was purified by CsCl centrifugation and translated in a reticulocyte cell-free system. By 4 h after infection, host mRNA is degraded or no longer functional, and predominantly immediate early products are obtained by cell-free translation (2). The polyacrylamide gel autoradiographs indicated that similar sets of polypeptides were made with both RNAs (Fig. 6). Thus, the deletion has no general effect on viral RNA synthesis.

The next step was to hybridize RNA from cells infected with standard or 6/1 virus to recombinant DNA containing either the *Eco*RI A, B, or C fragments of *Hin*dIII C. Specifically bound RNA was then translated, and the polypeptides were analyzed. The results (Fig. 6) can be summarized as follows: of those mRNA's encoded within the *Hin*dIII C fragment, only those from the *Eco*RI A portion were made in cells infected with variant 6/1. The apparent absence of the 21,000-molecular-weight (21K) polypeptide doublet encoded within *Eco*RI B suggests that its mRNA should be positioned slightly further to the right than indicated in Fig. 5. Thus, at least eight early mRNA's were not made. This is a minimum number since we have not yet completed mapping the last 1,500 nucleotides of *Hin*dIII C or the *Hin*dIII N fragment.

Conservation of the deleted DNA sequence in other poxviruses. The deletion had no apparent effect on the infectivity or yield of vaccinia virus (strain WR) in HeLa or BSC-1 cells (14). For that reason and because restric-

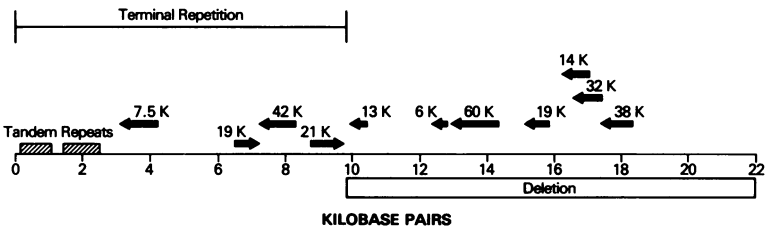

FIG. 5. *Translational map of the left end of the vaccinia virus genome. The location of the deletion occurring in variants 6/1 and 6/2 is indicated. Filled arrows indicate the size and direction of transcription of immediate early or early mRNA's. The number accompanying each arrow represents the size of the polypeptide translation product in kilodaltons. Map information was obtained from previous studies in this laboratory (3, 4, 24, 25).*

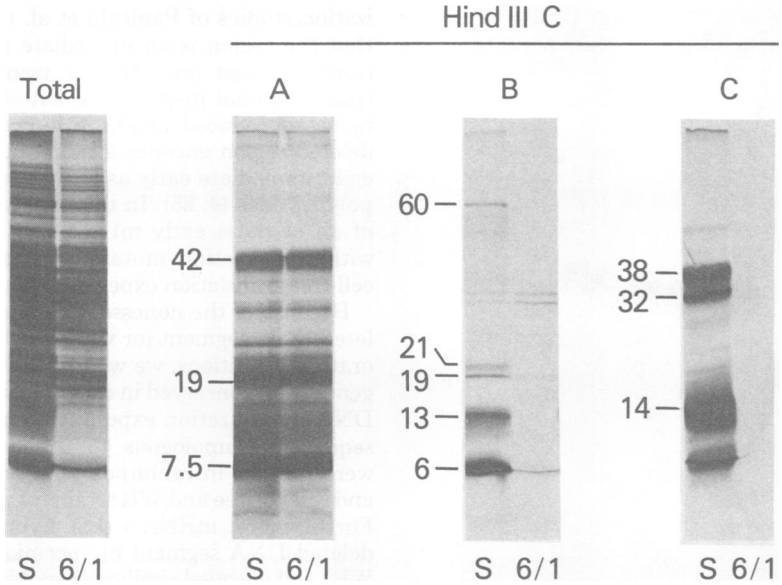

FIG. 6. *Autoradiograph of [^{35}S]methionine-labeled polypeptides resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Total or hybridization-selected cytoplasmic RNA from cycloheximide-treated cells infected with standard (S) or variant (6/1) virus was translated in a reticulocyte cell-free system. Recombinant DNAs containing either EcoRI A/HindIII C, EcoRI B/HindIII C, or EcoRI C/HindIII C were immobilized on filters and used for hybridization selection. Only polypeptides that have been mapped as distinct gene products are indicated. The numbers refer to the molecular weights of the polypeptides in thousands.*

tion endonuclease maps have shown considerable variation in the near terminal genome structure of orthopoxviruses (10), we wondered whether the deleted sequences were conserved in other viruses. Accordingly, the total genomes of vaccinia virus (strain WR), vaccinia virus (strain Elstree), rabbitpox (strain Utrecht), and cowpox (strain Brighton) were digested with *Eco*RI, and the electrophoretically separated fragments were transferred to a nitrocellulose membrane. A recombinant containing the *Eco*RI C fragment of *Hin*dIII C was chosen as the radioactive probe since that fragment has been entirely deleted from 6/1 and 6/2 variants of vaccinia virus (strain WR). Control experiments indicated that it hybridized to a single *Eco*RI fragment of standard vaccinia WR but to no fragments of 6/2 (Fig. 7). Significantly, the probe hybridized to single *Eco*RI fragments from each of the other viruses, indicating the presence of homologous sequences (Fig. 7). In vaccinia virus WR, vaccinia virus Elstree, rabbitpox, and cowpox, the sizes of the homologous *Eco*RI fragments were approximately 3.02, 3.93, 3.96, and 4.16×10^6 daltons, respectively.

To determine whether mRNA's are transcribed from the conserved *Eco*RI fragments, RNA from rabbitpox virus- and cowpox virus-infected cells was hybridized to recombinant DNA containing the *Eco*RI C fragment of *Hin*dIII C immobilized on a nitrocellulose filter. The specifically bound RNAs were then eluted and translated in the reticulocyte cell-free system. mRNA's for the 38K, 32K, and 14K polypeptides were made in rabbitpox virus-infected cells (Fig. 8). With cowpox RNA, the pattern of polypeptides was similar, but some differences in mobility and the apparent absence of the 32K polypeptide were noted (Fig. 8).

DISCUSSION

Previously, we described an unstable variant of vaccinia virus with a genome that appeared to undergo frequent recombinational events such that it consisted of a family of varying length molecules even after repeated virus plaque isolations (14). The reiterated portion of the genome was near the ends and included a set of 70-bp tandem repetitions. This isolate, as well as stable revertants, had a second apparently unrelated feature consisting of a large deletion. In the present communication, we have mapped the deletion rather precisely as starting 6.4×10^6 daltons from the left end of the genome, within or just beyond the small *Hin*cII E/*Eco*RI B/*Hin*dIII C fragment and ending within the *Hin*dIII N fragment. The deletion is more than 6×10^6 daltons long or 5% of the genome and

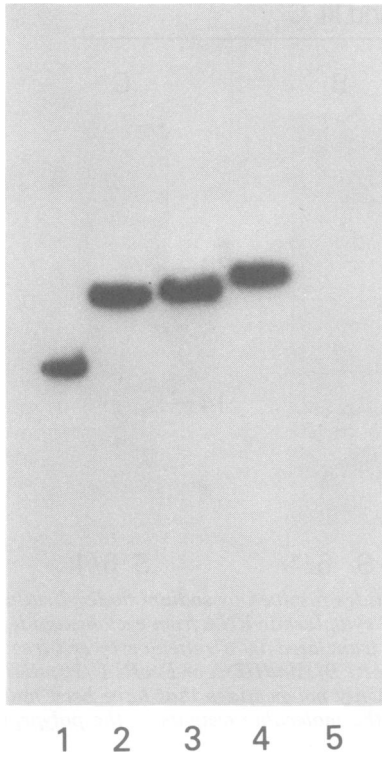

FIG. 7. *Autoradiograph of electrophoretically separated EcoRI fragments of various poxviruses hybridized to ^{32}P-labeled DNA. EcoRI digests of standard vaccinia virus strain WR (1), vaccinia virus strain Elstree (2), rabbitpox (3), cowpox (4), and 6/2 variant of vaccinia virus WR (5) were resolved by electrophoresis on a 1% agarose gel. The fragments were transferred to a nitrocellulose membrane and hybridized to ^{32}P-labeled recombinant DNA containing the EcoRI C/HindIII C fragment.*

starts very close to the junction of the inverted terminal repetition and unique sequence. The deletion described here appears to be very similar to the one recently reported by Panicalli et al. (17) in a stable "small DNA" variant. Indeed, we suspect that it is identical since their serially passaged stock was obtained originally from our laboratory.

The deletion within the vaccinia virus genome had no apparent effect on specific infectivity or virus yield in HeLa cells or plaque size in BSC-1 cells (14). Our preliminary experiments also indicated that variant 6/1 and 6/2 replicated in pig kidney cells (kindly supplied by R. Moyer) in contrast to the host range effects seen with some rabbitpox deletion mutants (16). Therefore, it was of particular interest to determine whether the deletion was in a silent or expressed region of the vaccinia virus genome. Blot hybridization studies of Panicalli et al. (17) suggested that the region is an immediate early or early transcriptional unit. Recent translational and transcriptional maps of the left side of the genome reproduced in Fig. 5 indicated that the deleted region encodes a minimum of seven or eight immediate early as well as two minor late polypeptides (4, 25). In this report, the absence of all of these early mRNA's in cells infected with the deletion mutant was established by cell-free translation experiments.

Because of the nonessential nature of the deleted DNA segment for virus growth under laboratory conditions, we wondered whether these genes were conserved in other poxviruses. DNA-DNA hybridization experiments indicated that sequences homologous to the deleted region were present in rabbitpox virus, cowpox virus, and the Elstree and WR strains of vaccinia virus. Furthermore, mRNA's that hybridized to the deleted DNA segment of vaccinia virus (strain WR) and encoded similar size polypeptides were made in cells infected with rabbitpox and cowpox viruses.

Although it may not be surprising that the large poxvirus genome encodes some nonessen-

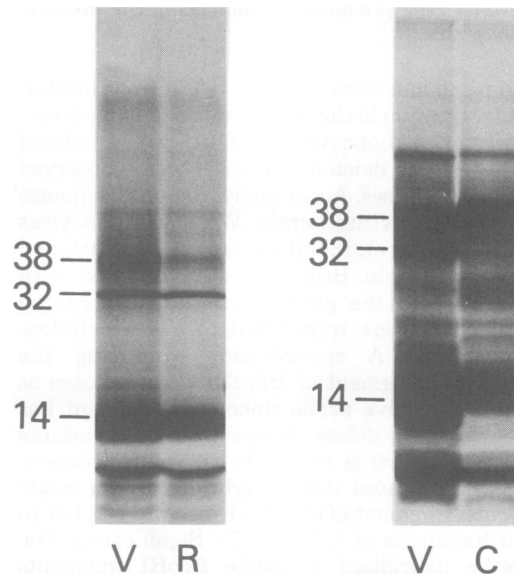

FIG. 8. *Autoradiography of [^{35}S]methionine-labeled polypeptides resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cytoplasmic RNA from cycloheximide-treated cells infected with standard vaccinia virus WR (V), rabbitpox virus (R), or cowpox virus (C) was purified by hybridization to recombinant DNA containing the EcoRI C/HindIII C fragment of vaccinia virus immobilized on a nitrocellulose filter. The selectively bound RNA was eluted and translated in a reticulocyte cell-free system.*

tial polypeptides, it is intriguing that they are encoded within a contiguous DNA segment. Perhaps the most interesting hypothesis is that genes located in this region have a related function. One function that is not required for virus replication in tissue culture cells is extracellular dissemination of virus particles. Studies by Payne (18) have demonstrated that the amount of extracellular virus formed varies widely in different vaccinia virus strains and that no correlation exists between the amount of virus released and total virus yield or plaque size. This apparent paradox is due to efficient cell-to-cell spread of vaccinia virus in tissue culture. The WR strain of vaccinia virus does not release significant amounts of extracellular virus, indicating that it already has lost some element necessary for this process. Thus, a further deletion would not be expected to have any additional deleterious effect. Experiments designed to test the above hypothesis regarding the function of the deleted genes have been initiated.

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