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Vaccinia virus encodes a polypeptide with DNA ligase activity

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ABSTRACT

Vaccinia virus gene SalF 15R potentially encodes a polypeptide of 63 kD which shares 30% amino acid identity with *S. pombe* and *S. cerevisiae* DNA ligases. DNA ligase proteins can be identified by incubation with α -(³²P)ATP, resulting in the formation of a covalent DNA ligase-AMP adduct, an intermediate in the enzyme reaction. A novel radio-labelled polypeptide of approximately 61 kD appears in extracts from vaccinia virus infected cells after incubation with α -(³²P)ATP. This protein is present throughout infection and is a DNA ligase as the radioactivity is discharged in the presence of either DNA substrate or pyrophosphate. DNA ligase assays show an increase in enzyme activity in cell extracts after vaccinia virus infection. A rabbit antiserum, raised against a bacterial fusion protein of β -galactosidase and a portion of SalF 15R, immune-precipitates polypeptides of 61 and 54 kD from extracts of vaccinia virus-infected cells. This antiserum also immune-precipitates the novel DNA ligase-AMP adduct, thus proving that the observed DNA ligase is encoded by SalF 15R.

INTRODUCTION

Vaccinia virus, the prototypical member of the poxvirus family, is a large DNA-containing virus which replicates in the cytoplasm of the host cell. The linear double-stranded genome of approximately 185,000 base pairs has the potential to encode at least 200 proteins (reviewed in 1). Although the nucleus is required for formation of mature virus particles, vaccinia virus DNA replication can occur in enucleated cells (reviewed in 2). The cytoplasmic site of replication requires that vaccinia virus encodes many enzymes and protein factors necessary for DNA synthesis, thus providing a unique system for molecular analysis of the functions associated with virus replication in a mammalian cell. Identification and analysis of such virus-encoded proteins may also provide valuable information on the enzymology of replication of cellular DNA.

A growing number of virus genes expressing proteins involved in DNA replication have been mapped on the vaccinia virus genome and sequenced. This comprises DNA polymerase (3), DNA topoisomerase (4) and a DNA-dependent ATPase (5). The enzymes of precursor biosynthetic pathways, thymidine kinase (6,7) thymidylate kinase (8) and the large and small subunits of ribonucleotide reductase (9,10) have also been characterised in this way. In addition, a gene has been identified in our laboratory which has extensive sequence homology to yeast DNA ligases (11). An increase in DNA ligase activity has been reported in vaccinia virus infected cells (12,13), although genetic evidence of virus origin was not obtained. Other replicative enzymes for which an increase in biochemical activity has been reported include single-strand specific nucleases (14) and nicking-joining enzyme (15).

DNA ligases join strand breaks in DNA by catalysing the formation of phosphodiester bonds, requiring a divalent metal ion and either ATP or NAD as cofactor. Studies on conditional lethal mutants of yeast, *E. coli* and bacteriophage T4 have established the involvement of DNA ligase in the replication, repair and recombination of DNA (reviewed in 16). The basic mechanism by which DNA ligase seals a DNA strand break is the same in mammalian cells as in bacteria. A DNA ligase-AMP covalent complex is formed, followed by transfer of the AMP moiety to the 5' terminus of the chain break, to generate a covaent DNA-AMP reaction intermediate prior to closure of the strand interruption. A substantial amount of biochemical information on mammalian DNA ligases has been obtained (reviewed in 17). Two immunologically unrelated proteins with DNA ligase activity, DNA ligases I and II, have been described, and may be responsible for DNA replication and repair, respectively. The molecular defect in the human genetic disease Bloom's syndrome is the result of a deficiency in DNA ligase I (18,19). However, the cloning and sequencing of a DNA ligase gene from a higher eukaryote have not yet been reported.

The SalI F fragment of Vaccinia virus DNA was sequenced and an open reading frame, SalF 15R, with the potential to encode a polypeptide of 63 kD was identified. Computerassisted database searches revealed extensive amino acid sequence homology with the DNA ligase genes of the yeasts *S. cerevisiae* and *S. pombe* (11). Here, biochemical and immunological analyses of DNA ligase in vaccinia virus-infected cells are presented. These experiments demonstrate that the polypeptide encoded by SalF 15R is a DNA ligase, a result which may lead to a better understanding of the mechanisms of vaccinia virus DNA replication and recombination.

MATERIALS AND METHODS

Cells and Virus

African green money kidney (CV-1) cells and human TK^- 143 cells were maintained in Glasgow modified Eagle's medium (GMEM) containing 10% foetal bovine serum. Vaccinia virus strain WR was grown in BHK cells and titrated on CV-1 cells (20).

Chromatography and Buffers

DE52 ion-exchange cellulose (Whatman) was prepared according to the manufacturers instructions. P11 phosphocellulose (Whatman) was prepared as described (21). All sodium chloride buffers were at pH 7.5 and contained 50 mM Tris-HCl, 1 mM EDTA and 10 mM 2-mercaptoethanol.

Extraction of DNA ligase

All operations were carried out at 4°C. CV-1 cells in monolayer culture, 1×10^7 cells per sample either mock infected or infected with 100 p.f.u./cell vaccinia virus, were collected by scraping with a rubber policeman and centrifugation, then washed in phosphate-free cell culture medium. Cells were harvested at the times post-infection (p.i.) indicated in the presence or absence of cytosine arabinoside (40 µg/ml). The cell pellet was resuspended in 1 ml ice-cold extraction buffer (0.1 M NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM 2-mercapto-ethanol, 1 mM EDTA, 23 µg/ml aprotinin, 0.5 µg/ml each of pepstatin, leupeptin and chymostatin) and disrupted in a glass hand homogeniser, as described in (18). In some experiments, DNA ligase was partially purified by a two column procedure. The Dounce homogenate was centrifuged to remove cell debris then the supernatant made 0.4 M NaCl and passed through a DE52 column equilibrated with 0.4 M NaCl buffer. Fractions containing protein (measured by the Bradford assay (22)) were pooled and dialysed against 50 mM NaCl buffer. The dialysed samples were loaded onto a phosphocellulose column, equilibrated with 50 mM NaCl buffer. The column was washed with three column



Fig. 1: Identification of vaccinia virus DNA ligase protein. Crude extracts were prepared from mock infected or vaccinia virus infected (100 pfu/cell) CV1 cells by Dounce homogenisation in 100 mM NaCl buffer as described in Materials and Methods. Vaccinia virus infected early (lane 2), late (lane 3) or mock infected (lane 1) CV1 cell extracts and purified calf thymus DNA ligase I (a gift from T. Lindahl) (lane 5) were incubated with α -(³²P) ATP (Methods). Reactions were terminated by trichloroacetic acid and covalently labelled polypeptides analysed by SDS PAGE on a 12.5% gel.

volumes of the same buffer, then enzyme activity eluted with 1 M NaCl buffer. The resulting fractions were dialysed against 50 mM NaCl buffer containing 50% glycerol and stored at -20° C prior to assays of DNA ligase activity, formation of DNA ligase-AMP adducts, and immune-precipitation. DNA ligase was also analysed in crude extracts without column fractionation. In this case, cells were harvested as above and after Dounce homogenisation and centrifugation at 10K for 20 minutes at 4°C the supernatant and pellet fractions were assayed directly.

Assay of DNA Ligase Activity

DNA ligase was assayed using a poly dA. oligo 5'-(³²P)-dT substrate by a modification of the protocol described in (23). Incubations were at 16°C for 15 minutes and the reaction was stopped and substrate denatured by heating to 90°C for 10 minutes. The samples were extracted with phenol:chloroform, precipitated in ethanol then resuspended in water. Formamide dye mix was added and the samples electrophoresed on a 12% acrylamide DNA sequencing gel using standard procedures (24).

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Formation of DNA ligase-AMP adduct

DNA ligase was radioactively labelled by incubating cell extracts with α -(³²P) ATP followed by TCA precipitation essentially as described in (25), except that incubations were at room temperature for 30 minutes. DNA ligase-AMP adducts were visualised by 12.5% SDS polyacrylamide gel electrophoresis and autoradiography of the dried gel. *Antisera and Immune-Precipitations*

A plasmid, pSK12, expressing a β -galactosidase/Sa1F 15R fusion protein was constructed by cloning the 550 nucleotide BgIII fragment from Sa1F 15R DNA into the BamHI site of pEX3 (26). The BgIII sites are at positions 516 and 1066 in the fragment of SalF presented in (11). On induction of bacterial cultures containing pSK12, a β -galactosidase fusion protein of the predicted Mr was detected by SDS-PAGE and staining with Coomassie brilliant blue. After gel purification and electroelution, 250 μ g aliquots of this protein were used to immunise a rabbit resulting in the production of pEX LIG antiserum.

TK⁻ cells infected at 30 p.f.u./cell with either wild-type vaccinia virus, or mock infected, were incubated in methionine-free medium from 1 to 1.5h post infection, then radiolabelled by incubation in 100 μ Ci/ml of (³⁵S)-methionine in MEM without methionine for 2.5h. Immune precipitation was as described in (27). The cells were lysed in RIPA buffer (50 mM Tris-HCl pH 7.2, 150 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, 1% Triton X-100) plus 5 μ g/ml DNase and 1 mM phenylmethylsuphonyl fluoride for 15 min on ice. Lysates were clarified by centrifugation at 10,000 r.p.m. for 15 min at 4°C. Supernatants were incubated with rabbit sera for 30 min at room temperature and immune complexes precipitated by incubation with 50 μ g of a 50% suspension of Protein A-Sepharose for 2h at 4°C. The beads were washed twice with NP40 buffer and once with 2 M urea, 0.4 M LiCl, 10 mM Tris-HCl pH 8.0, then SDS sample buffer added and proteins analysed by SDS-PAGE.

In some experiments, unlabelled cell lysate fractions containing DNA ligase were incubated with α -(³²P) ATP to form a DNA ligase-AMP adduct then immune-precipitated and analysed as described above.

RESULTS

Identification of the Vaccinia Virus DNA Ligase Protein

A feature of the reaction mechanism of all DNA ligases is the intermediate formation of a stable DNA ligase-AMP adduct. An assay making use of this feature has been developed by which DNA ligase polypeptides can be identified by incubation with α -(³²P) ATP (25). The type of covalent linkage formed is rare among other proteins, therefore the assay is suitable for use on relatively crude preparations and was used to determine whether a vaccinia virus DNA ligase could be detected.

Extracts from vaccinia virus infected cells contain a novel radio-labelled polypeptide of molecular weight approximately 61 kD after incubation with α -(³²P) ATP (Figure 1). This activity is detectable in both crude and partially purified extracts, at early (lane 2) and late (lane 3) times post infection. The size estimated by SDS-PAGE is in good agreement with that predicted from the amino acid composition of SalF 15R, 63 kD (11), which would be consistent with a lack of extensive post-translational modification. The extent of incorporation of radioactivity is much greater than that in mock infected cells, in which only a faint band of approximately 46 kD is visible (lane 1). This polypeptide is also present at reduced intensity in extracts from vaccinia virus infected cells. The 130 kD mammalian DNA ligase I, highly purified from calf thymus, is shown in lane 5. Mock infected cells



Fig. 2: The 61 kD polypeptide is a DNA ligase. A DNA ligase preparation partially purified from vaccinia virus infected cells late (15h) post infection was labelled with α -(³²P) ATP (lane 3). Preparations of calf thymus DNA ligase (a gift from T. Lindahl, ICRF) (lane 1) and bacteriophage T4 DNA ligase (New England Biolabs) (lane 2) were labelled in parallel. The vaccinia sample was divided into four equal parts. One part was analysed without further manipulation (lane 3) and the remainder centrifuged through a column to remove unincorporated ATP as described in (25) except that Sephadex-G25 was used. The excluded volume was divided into three equal parts and incubated at 37°C for 30 minutes with either no addition (lane 4), cold poly (dA):oligo (dT) DNA ligase substrate (lane 5) or 100 μ M sodium pyrophosphate (lane 6). The products were analysed as in Figure 1.

contain no polypeptide which co-migrates with the 61 kD band in infected cell extracts, suggesting the appearance of this protein is a consequence of infection with vaccinia virus.

The 61 kD polypeptide has the properties expected of a DNA ligase (Figure 2). A phosphocellulose column fraction derived from extracts of vaccinia virus infected cells late in infection was incubated with α -(³²P) ATP and then excess ATP was removed using Sephadex G-25. The excluded protein was incubated with either no addition (lane 4), DNA ligase substrate (lane 5) or sodium pyrophosphate (lane 6). The presence of DNA substrate allows the ligase reaction to proceed to completion, with a disappearance of (³²P)-AMP from the enzyme. Conversely, high concentrations of pyrophosphate drive the equilibrium back towards free enzyme and ATP, again with a consequent discharge of radioactivity from the polypeptide (Figure 2). This result strongly suggests that the 61 kD polypeptide is a DNA ligase with DNA strand joining activity.



Fig. 3: DNA ligase activity in vaccinia virus infected cells. Crude extracts from CV-1 cells infected with vaccinia virus early (3h), late (17h) post infection or mock infected were assayed for DNA ligase activity (Methods). The 30mer (32 P) oligo dT:poly dA substrate is shown in lane 1 and corresponds to the monomer n = 1. Four units (lane 2), 0.4 units (lane 3) and 0.04 units (lane 4) of bacteriophage T4 DNA ligase (New England Biolabs) were assayed in parallel and provide markers (n = 2, n = 3, n = 4). Lanes 5, 6 and 7 represent the supernatant

DNA Ligase Activity in Vaccinia Virus Infected Cells

An assay which measures ligation of 30 mer (32 P)-dT oligodeoxynucleotides annealed to poly dA was used to determine whether an increase in DNA ligase activity could be detected upon vaccinia virus infection. Activity is represented by the appearance of labelled products corresponding to two ligated molecules of the dT oligonucleotide (n=2), trimers of dT (n=3) and further higher oligomers. The assay of bacteriophage T4 DNA ligase provides a standard for this activity. An increase in DNA ligase activity above the basal level measurable in mock infected cells is observed after vaccinia virus infection (Figure 3). The ligase activity early in infection (lanes 5 and 8) is only slightly greater than the cellular activity in mock infected cells (lanes 6 and 9), but by late in infection (lanes 7 and 10) the activity is substantially higher. Extracts prepared in this low salt extraction buffer (100 mM NaCl, Methods) have an appreciable portion of the total DNA ligase activity located in the pellet fraction after centrifugation (lanes 8–10) compared to the supernatant (lanes 5–7) but an increase in the salt concentration to 1 M shifts the majority of the total DNA ligase activity into the soluble fraction (data not shown).

Immunological Analysis of Vaccinia Virus DNA Ligase

Approximately one third (183 amino acids) of the protein encoded by SalF 15R was cloned into the bacterial expression vector pEX3 (Methods). A rabbit polyclonal antiserum (pEX LIG) was raised against the resulting β -galactosidase/SalF 15R fusion protein. The pEX LIG antiserum immune-precipitates two virus polypeptides from extracts of cells labelled with (³⁵S)-methionine 1.5–4h post infection (Figure 4A, lane 2). The upper band, of molecular weight approximately 61 kD on SDS-PAGE, is more intense than the lower, of approximately 54 kD. No protein is recognised in mock infected cells (lane 1). The portion of the gene inserted into the pEX LIG construct does not include the regions of strongest amino acid sequence homology with yeast DNA ligases, therefore cross-reaction with mammalian ligases might not be expected. Both polypeptides are early virus gene products as treatment of the cells with cytosine arabinoside, an inhibitor of DNA replication, does not affect their expression (data not shown). De novo synthesis of both proteins can be detected early in infection by pulse labelling with (³⁵S) methionine 1.5–2.5h post infection followed by immune-precipitation, but only slightly reduced levels are observed as late as 7–8h p.i. (data not shown).

The larger of the two polypeptides detected by immune-precipitation with the pEX LIG antiserum co-migrates with the DNA ligase-AMP adduct from vaccinia virus infected cells on SDS-PAGE (Figure 4B). The marginal difference in size between the (³²P) and (³⁵S)-labelled polypeptides which may be detected on electrophoresis to achieve maximum resolution is possibly due to the addition of the AMP moiety in the (³²P)-labelled protein. The identity of the lower band is not clear. Pulse-chase experiments have not indicated a precursor-product relationship (data not shown). It is possible that the 54 kD polypeptide represents the translation product of the minor mRNA of SaIF 15R, detected by S1 mapping (11), but this is unlikely due to the relative ratios of the 54 and 61 kD proteins versus the shorter and full length RNAs.

SalF 15R Encodes the Vaccinia Virus DNA Ligase

The DNA ligase would be likely to be an essential gene if it was involved in DNA replication, in which case it would not be possible to select recombinant virus containing

fractions from early, mock and late samples respectively after Dounce homogenisation and centrifugation at 10K for 20 minutes. Lanes 8, 9 and 10 are the pellet fractions from early, mock and late samples. An autoradiograph of the dried gel is shown.

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Fig. 4: A. Immune-precipitation of $({}^{35}S)$ -methionine labelled polypeptides from vaccinia virus infected cells. TK⁻ cells infected with vaccinia virus (30 pfu/cell) or mock infected were labelled with $({}^{35}S)$ -methionine 1.5–4h post infection. Cell extracts were prepared and immune-precipitated with pEX LIG antiserum (Methods). Lane I represents uninfected cells and lane 2 vaccinia virus infected cells. Molecular weight markers are shown to the right of the gel. B. Co-migration of $({}^{35}S)$ -methionine and α - $({}^{32}P)$ -ATP labelled proteins. A $({}^{32}P)$ -labelled DNA ligase-AMP adduct from vaccinia virus infected cells (lane 1) and cell extracts labelled with $({}^{35}S)$ -methionine 2.5–6h p.i. from either vaccinia virus infected (lane 2) or mock infected cells (lane 3), immune-precipitated with pEX LIG antiserum as described in Part A, were electrophoresed through a 12.5% polyacrylamide gel.

a specific deletion of this gene. An alternative approach to prove that SalF 15R gene product was responsible for the increased DNA ligase activity in vaccinia virus infected cells was therefore chosen. This made use of the pEX LIG antiserum, raised against SalF 15R encoded protein, in immune-precipitation experiments against the radiolabelled DNA ligase-AMP adduct. The pEX LIG antiserum can efficiently precipitate the (³²P)-labelled DNA ligase protein in extracts from vaccinia virus infected cells (Figure 5, lane 5), whereas pre-immune serum from the same rabbit (lane 4), or a non-specific immune serum raised against an unrelated pEX fusion protein (lane 6), do not recognise the 61 kD polypeptide (Figure 5). Control experiments indicate that neither purified calf thymus (lane 1) nor bacteriophage T4 DNA ligase (lane 2) can be immune-precipitated by the pEX LIG antiserum (data not shown). The immune-precipitation of the novel DNA ligase-AMP adduct by the antiserum



Fig. 5: Immune-precipitation of labelled vaccinia virus DNA ligase. Calf thymus DNA ligase (lane 1), T4 DNA ligase (lane 2) and a phosphocellulose column fraction from vaccinia virus infected cells (lane 3) were incubated with α -(³²P)-ATP (Methods). Each sample was divided into four equal parts and either analysed directly by TCA precipitation and SDS-PAGE (lanes 1, 2 and 3) or, in the case of extract from vaccinia virus infected cells, immune-precipitated with either pre-immune serum (lane 4), pEX LIG serum (lane 5) or a non-specific pEX immune serum (lane 6), followed by SDS-PAGE.

raised against SalF 15R encoded protein clearly demonstrates that this vaccinia virus gene encodes the observed DNA ligase activity.

DISCUSSION

A vaccinia virus gene encoding a predicted polypeptide of 63 kD which shares 30% amino acid sequence identity with *S. cerevisiae* and *S. pombe* yeast DNA ligases has been identified (11). In this paper the protein encoded by this gene has been detected and shown to have the biochemical characteristics of a DNA ligase. The extensive amino acid sequence homology to yeast DNA ligases, increase in DNA ligase activity in vaccinia virus infected cells and appearance of a novel DNA ligase-AMP adduct of approximately the predicted molecular weight all strongly suggest that SalF 15R encodes this enzyme. Immune-precipitation of the DNA ligase-AMP adduct by antiserum raised against SalF 15R encoded protein proves that this is indeed the case.

The ability to label the vaccinia virus DNA ligase by incubation with α -(³²P) ATP shows that in common with eukaryotic DNA ligases and bacteriophage DNA ligases, ATP is utilised in the reaction. In contrast, the DNA ligases of *E. coli*, *B. subtilis and S.*

typhimurium are NAD-requiring. The lysine residue which forms the covalent enzyme-AMP linkage is likely to be amino acid number 382, by alignment with that previously identified in yeast DNA ligases (11). It will be of interest to determine whether the properties of vaccinia virus DNA ligase correspond more closely to the type I or type II mammalian enzymes. The type I enzyme can catalyse blunt-end joining of DNA, increases in rapidly dividing cells during rat liver regeneration and is the major ligase of DNA replication (reviewed in 28). DNA ligase II is involved in DNA repair processes, and unlike type I can use hybrid DNA:RNA molecules as substrate, allowing the development of typespecific assays (23). It will be possible to assess the biochemical properties of vaccinia virus DNA ligase, such as pH and temperature optima and salt requirements, after purification assisted by high level expression of the gene in a suitable vector system.

A substantial increase in DNA ligase activity, measured by joining synthetic dT oligonucleotides annealed to poly dA, is observed after vaccinia virus infection. This result is consistent with the increase in DNA ligase activity reported twenty years ago (12). In that study, up to a 13-fold increase was measured early in infection and was not inhibited by cytosine arabinoside, an inhibitor of DNA replication and therefore late gene expression. The level of activity late in infection was not reported. Sambrook and Shatkin (12) did not detect activity in purified virions after detergent solubilisation, a result also obtained in (13). The more sensitive assay for DNA ligase-AMP adduct formation described here was also unable to detect DNA ligase activity packaged within the virion core (data not shown). DNA ligase is the product of an early gene, therefore the activity will be present prior to the onset of DNA replication. However, the enzyme activity increases with time, as there is a greater level of enzyme activity late in infection than early. This is somewhat surprising in view of the small amount of SalF 15R-specific RNA detectable late (10h) post infection (11) and may be due in part to protein synthesised early in infection remaining stable.

The identification of a gene encoding DNA ligase proves that the increase in DNA ligase activity is of viral origin. This need not have been the case, as vaccinia virus replication models exist for which a DNA ligase is not required. Furthermore, even if such an activity was necessary, it is possible that rather than encoding its own DNA ligase, vaccinia virus could cause the transport of cellular DNA ligase from the nucleus, or induce a cellular DNA ligase gene. There would be some precedent for this, as the large subunit of RNA polymerase (29) and a cellular lamin (30) are both recruited to the cytoplasm upon vaccinia virus infection. Cellular DNA ligase I is known to be susceptible to proteolysis, possibly due to a domain structure with protease-sensitive hinge regions. The intact mammalian enzyme has a molecular weight of 200 kD, which is rapidly cleaved to a 130 kD fragment and more slowly to an 85-90 kD active fragment (17; see also Figure 2, lane 1). DNA ligase II has a molecular weight of approximately 80 kD. A mammalian DNA ligase fragment of 61 kD has not, however, been reported.

Although much progress has been made in unravelling the overall features of the mechanism of vaccinia virus DNA replication (reviewed in 31) several possible models exist. After infection, nicks are introduced near one or both of the ends of the genome and DNA replication starts via self-priming by the 3' ends generated by the nicks. This initial self-priming alone, followed by a strand displacement mechanism, could account for DNA synthesis along the entire genome leading to the generation of concatameric structures. DNA ligase would not be required, as a nicking-joining (NJ) enzyme capable of first introducing and then sealing nicks at the ends of the genome has been described

(15) and would be capable of resolving concatameric molecules into monomers. This enzyme, which is probably encoded by vaccinia virus although the gene has not been identified, is distinct from the DNA ligase described here on the basis of its smaller molecular size (50 kD processed to 44 kD), ATP independence and concerted mode of action, cleaving superhelical DNA site-specifically followed by joining to form cross-links. Furthermore, nicks introduced by DNase I treatment, equivalent to the poly(dA):oligo(dT) DNA ligase substrate, are not joined by NJ enzyme (32). NJ enzyme is also distinct from the vaccinia virus type I DNA topoisomerase, which has a nicking-closing activity (33).

A second possible model is that after self-priming at the genome ends, DNA replication proceeds bi-directionally via leading and lagging strand synthesis, with formation of RNA primers and Okazaki-type fragments of DNA. Concatamers could again be formed, and some evidence for this mechanism does exist (e.g. 34,35). A central requirement would be for a DNA ligase to join the short DNA fragments on the lagging strand. The construction of conditional viral mutants in the SalF 15R gene will allow these models and the various functions of the DNA ligase in vaccinia virus DNA replication and recombination to be investigated.

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