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Identification of Herpes Simplex Virus Type 1 Genes Required for Origin-Dependent DNA Synthesis

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The herpes simplex virus (HSV) genome contains both *cis*- and *trans*-acting elements which are important in viral DNA replication. The *cis*-acting elements consist of three origins of replication: two copies of ori_S and one copy of ori_L. It has previously been shown that five cloned restriction fragments of HSV-1 DNA together can supply all of the *trans*-acting functions required for the replication of plasmids containing ori_S or ori_L when cotransfected into Vero cells (M. D. Challberg, Proc. Natl. Acad. Sci. USA, 83:9094–9098, 1986). These observations provide the basis for a complementation assay with which to locate all of the HSV sequences which encode *trans*-acting functions necessary for origin-dependent DNA replication. Using this assay in combination with the data from large-scale sequence analysis of the HSV-1 genome, we have now identified seven HSV genes which are necessary for transient replication of plasmids containing either ori_S or ori_L. As shown previously, two of these genes encode the viral DNA polymerase and single-stranded DNA-binding protein, which are known from conventional genetic analysis to be essential for viral DNA replication in infected cells. The functions of the products of the remaining five genes are unknown. We propose that the seven genes essential for plasmid replication comprise a set of genes whose products are directly involved in viral DNA synthesis.

Herpes simplex virus (HSV) contains a large (150 kilobase [kb]) double-stranded DNA genome which is replicated within the nucleus of the host cell (4). Although the mechanistic details of HSV DNA synthesis are largely unknown, it is clear from the work of several laboratories that the HSV genome contains both *cis*- and *trans*-acting elements which are important in viral DNA replication. Thus, dissection of this process should be amenable to the combination of biochemistry and genetics which has proven so powerful in other systems.

The existence of *cis*-acting replication origins was first inferred from the structure of defective viral genomes (46, 50). It is now thought that there are three such origins and that they consist of two distinct but related DNA sequences (46–48, 50, 52): ori_L, of which there is one copy in the viral genome, and ori_S, of which there are two copies. Plasmid DNAs containing either ori_S or ori_L are replicated when they are introduced into HSV-infected cells (46–48, 50, 52). On the basis of this result, it seems reasonable to assume that ori_S and ori_L are sites at which viral DNA synthesis is initiated, although direct evidence to support this assumption is not yet available.

Two complementary approaches have been used to identify *trans*-acting viral gene products involved in the replication of viral DNA. First, cell-free extracts have been assayed for virus-induced biochemical activities which, by analogy to other, better-defined replication systems, probably have a role in DNA replication. Such biochemical activities identified in extracts of HSV-infected cells include a DNA polymerase (Pol) (23), a single-strand-specific DNA binding protein (DBP) (3, 41), an origin-specific DBP (17), a thymidine kinase (25), a ribonucleotide reductase (RR) (12), and an exonuclease (23, 31). Second, several virus mutants have been isolated which exhibit alterations in DNA synthesis (8, 9, 11, 16, 24, 29, 37, 44, 49). Such mutants include members of at least 10 different complementation groups. In several cases these two approaches have converged; mutants defective in DNA replication with lesions mapping within the coding sequences for Pol (8, 11, 20), DBP (13, 43, 51), RR (40; S. K. Weller, personal communication), and possibly the exonuclease (23, 33) have been identified.

To overcome some of the limitations of these two approaches, a third approach based on the transient replication of transfected plasmid DNAs has recently been developed. We have reported that five cloned restriction fragments of HSV DNA together can supply all of the functions required for the replication of plasmids containing ori_S or ori_L when cotransfected into Vero cells (7). Subcloning of one of these fragments, *Xba*I-F, revealed that the genes for two proteins known to be required for viral DNA replication, DBP and Pol, were similarly required for plasmid replication in the transient assay system. These observations provide the basis for a complementation assay with which to locate all of the HSV sequences which encode *trans*-acting functions necessary for origin-dependent DNA replication.

In this paper we report the results of such an analysis. We subcloned each of the large plasmids required for transient DNA synthesis and assayed each subclone, alone and in combinations, for the ability to substitute for the original clone. This approach, when combined with data from large-scale DNA sequence analysis of the HSV-1 genome, allowed us to identify five genes in addition to *dbp* and *pol* which are essential for origin-dependent DNA replication. The sequences of these five genes are reported in the accompanying paper (30).

MATERIALS AND METHODS

Construction and isolation of plasmids. The structure and source of all plasmids used in this work are summarized in Table 1. The fragment of viral DNA in each of these plasmids was derived originally from the KOS strain of HSV-1. The DNA sequence analyses from which the restric-

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TABLE 1. Plasmids used

Plasmid	Map coordinates	HSV gene[a]	Reference
pSG25 (*Eco*RI-H)	0.87–0.97		21
pKB-X (*Bam*HI-X)	0.94–0.96		S. K. Weller, unpublished
pMC121 (*Xba*I-C)	0.07–0.29		7
pCW8	0.07–0.11		This work
pCW21	0.08–0.11	UL5	This work
pCW9 (*Kpn*I-F)	0.12–0.19		This work
pMC160	0.12–0.16		This work
pMC160-1	0.12–0.16	UL9	This work
pMC160-2	0.12–0.16	UL8	This work
pCW16	0.16–0.19		This work
pMC122 (*Xba*I-F)	0.29–0.45		7
pNN1	0.38–0.42	*dbp*	7
pNN3	0.40–0.44	*pol*	7
pMC123 (*Xba*I-E)	0.45–0.64		7
pKH-F (*Hpa*I-F)	0.54–0.62		S. K. Weller, unpublished
pCW15	0.59–0.64		This work
pNN4	0.61–0.63	UL42	This work
pMC124 (*Xba*I-D)	0.64–0.83		7
pKB-L (*Bam*HI-L)	0.72–0.74		This work
pNN5	0.72–0.74	UL52	This work
pSG1 (*Eco*RI-JK)	0.0–0.086, 0.83–0.87		21
pKB-B (*Bam*HI-B)	0.74–0.80		This work
pMC150	0.75–0.77	IE63	This work
pMC151	0.80–0.82	IE110	This work
pK1-2	0.83–0.86	IE175	15
pKB-F (*Bam*HI-F)	0.64–0.70		This work

[a] Refers to the HSV gene in those plasmids containing a single intact gene.

tion maps and arrangement of genes displayed in Fig. 1 to 5 were deduced were performed on HSV-1 strain 17. Strain KOS and strain 17 are closely similar, and every restriction site indicated in the figures was shown to be present as indicated for the cloned KOS DNA. All of the plasmids constructed during the course of this work contain HSV fragments derived from the indicated *Xba*I clone which were inserted by standard methods (28) into the multiple cloning site of Bluescribe, a 3.2-kb cloning vector obtained from Vector Cloning Systems, San Diego, Calif. Plasmid DNAs were isolated from the DH5 strain of *Escherichia coli* by the method of Birnboim and Doly (5) and were further purified by banding in cesium chloride-ethidium bromide gradients.

Assay for plasmid DNA replication. Plasmid DNA replication was assayed exactly as described previously (7). Briefly, combinations of plasmid DNAs (0.5 μg of each) were mixed and used to transfect confluent monolayers of Vero cells by the calcium phosphate coprecipitation technique (22). At 14 to 16 h following transfection, the cells were lysed and total cellular DNA was isolated. The purified DNA was digested with *Eco*RI, *Hin*dIII, and *Dpn*I and fractionated by agarose gel electrophoresis. The DNA in the gel was transferred to a nylon membrane and probed with ^{32}P-labeled pUC19 DNA.

RESULTS

Our standard assay for HSV origin-dependent DNA replication used the following plasmids: pK1-2, encoding the immediate-early protein IE175; pMC122 (*Xba*I-F), which contains *dbp*, *pol*, and ori_L; pSG25, which contains two copies of ori_S but no essential *trans*-acting genes; and pMC121 (*Xba*I-C), pMC123 (*Xba*I-E), and pMC124 (*Xba*I-D), each of which contains at least one unidentified *trans*-acting gene. DNA replication was assayed by transfecting Vero cells with a mixture of these six plasmids. At 14 to 16 h following transfection, total cellular DNA was isolated, digested with *Eco*RI, *Hin*dIII, and *Dpn*I, fractionated by agarose gel electrophoresis, transferred to a nylon membrane, and probed with ^{32}P-labeled pUC19 DNA. Under these conditions, ori_L-dependent replication gives rise to a *Dpn*I-resistant fragment of 2.7 kb derived from *Xba*I-F, and ori_S-dependent replication gives rise to a *Dpn*I-resistant fragment of 4.6 kb derived from pSG25 (7).

To locate all of the unidentified genes required for plasmid DNA replication in our assay, we used the following general strategy. *Xba*I-C, *Xba*I-E, or *Xba*I-D was digested with one of a number of different restriction enzymes, and the resulting fragments were tested for their ability to substitute for the intact plasmid in a standard replication assay. Once we found a restriction enzyme which did not inactivate a plasmid, we cloned each of the fragments produced by that enzyme and tested the subclones, alone and in combinations, for their ability to supply the required function(s). This process was repeated until we obtained an active subclone containing a limited number of genes, as deduced by DNA sequence analysis. Finally, plasmids containing single intact genes were constructed and tested in a similar fashion. The results of these analyses are displayed in Fig. 1 to 5 and described in detail below.

***Xba*I-C.** Preliminary experiments showed that digestion of *Xba*I-C with *Kpn*I did not reduce its ability to support ori_L or ori_S-dependent replication. Accordingly, several *Kpn*I fragments were cloned and tested for their ability to substitute for *Xba*I-C. A combination of two of these subclones, pCW8 and pCW9, but neither of these two plasmids alone supported DNA replication at a level comparable to that seen with *Xba*I-C (Fig. 1B and D). Therefore, there must be at least two essential genes in *Xba*I-C. The essential gene(s) in pCW9 was further localized by dividing pCW9 roughly in half into plasmids pMC160 and pCW16. A combination of pMC160 and pCW8 completely complemented assay mix-

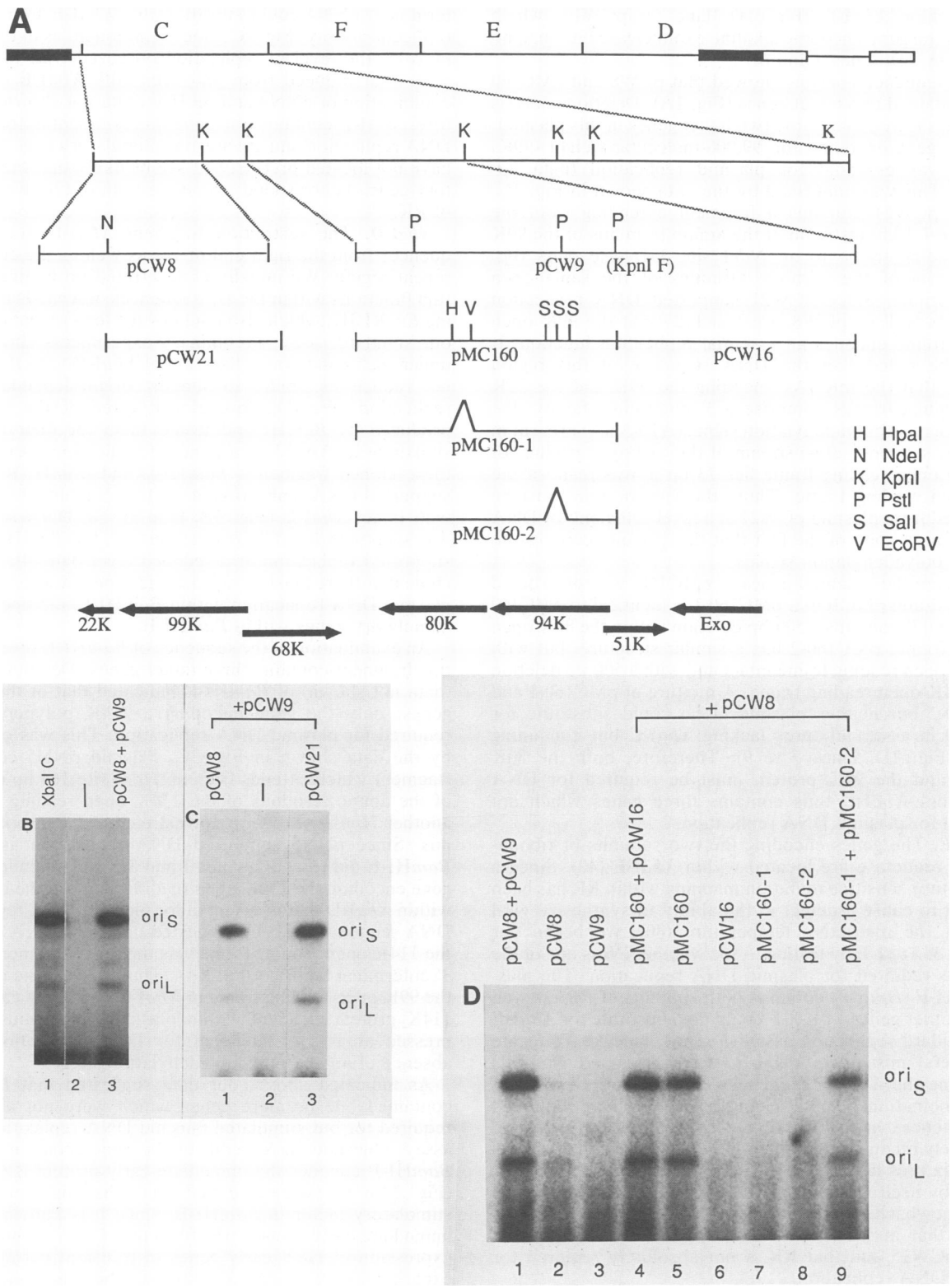


FIG. 1. Identification of DNA replication functions in *Xba*I-C. (A) Diagramatic representation of the HSV sequences contained within plasmid subclones of *Xba*I-C, shown with respect to the P arrangement of the HSV genome. The bold arrows at the bottom depict the arrangement of open reading frames deduced from DNA sequence data (30; D. J. McGeoch, unpublished data). Open and solid bars indicate the short inverted repeat and the long inverted repeat sequences, respectively. Exo, Exonuclease. (B, C, and D) DNA replication assays. Vero cells were transfected with 0.5 μg each of pMC122 (*Xba*I-F), pMC123 (*Xba*I-E), pMC124 (*Xba*I-D), pK1-2 (IE175), pSG25, and the plasmid(s) indicated at the top of each lane. ori_S and ori_L refer to the positions of *Eco*RI-*Hin*dIII-digested pSG25 and pMC122, respectively, which were run as markers on the same gel.

tures lacking *Xba*I-C (Fig. 1D, lane 5); pCW16, which contains the gene encoding alkaline exonuclease (36), had no effect (Fig. 1D, lanes 4 and 6).

DNA sequence analysis showed that pCW8 and pMC160 each contained two intact genes (Fig. 1A). Deletion analysis of pCW8 showed that of the two intact genes in this plasmid, only the gene encoding the 99,000-molecular-weight (99K) protein was essential for plasmid replication (data not shown). This was confirmed by the data shown in Fig. 1C. Plasmid pCW21 contains a fragment extending from the *Kpn*I site 1.1 kb upstream of the amino terminus of the 99K open reading frame to an *Nde*I site 351 base pairs (bp) downstream of the carboxy terminus of the same open reading frame. Since pCW21 supported DNA replication equally as well as pCW8, we conclude that the 99K open reading frame encodes an essential replication function. It should be noted that the DNA sequence of this region suggests that the mRNAs encoding the 99K and the 22K open reading frames comprise a 3′ coterminal family: the first consensus polyadenylation signal (ATTAAA) present in the DNA sequence downstream of the carboxy terminus of the 99K open reading frame lies 73 bp downstream of the 22K open reading frame. Thus, the 99K protein must be expressed from plasmid pCW21 at a level sufficient for DNA replication in spite of the fact that this plasmid contains no obvious polyadenylation signals.

To locate the essential genes in pMC160, we constructed pMC160-1 and pMC160-2. pMC160-1 is identical to pMC160 except that it contains a 500-bp deletion within the 80K open reading frame; pMC160-2 has a similar structure, but with the 80K open reading frame intact and with a 500-bp deletion in the 94K open reading frame. A mixture of pMC160-1 and pMC160-2 but neither plasmid alone could substitute for pMC160 in assay mixtures lacking *Xba*I-C but containing pCW8 (Fig. 1D, lanes 7 to 9). Therefore, both the 80K protein and the 94K protein must be required for DNA replication. *Xba*I-C thus contains three genes which are essential for plasmid DNA replication.

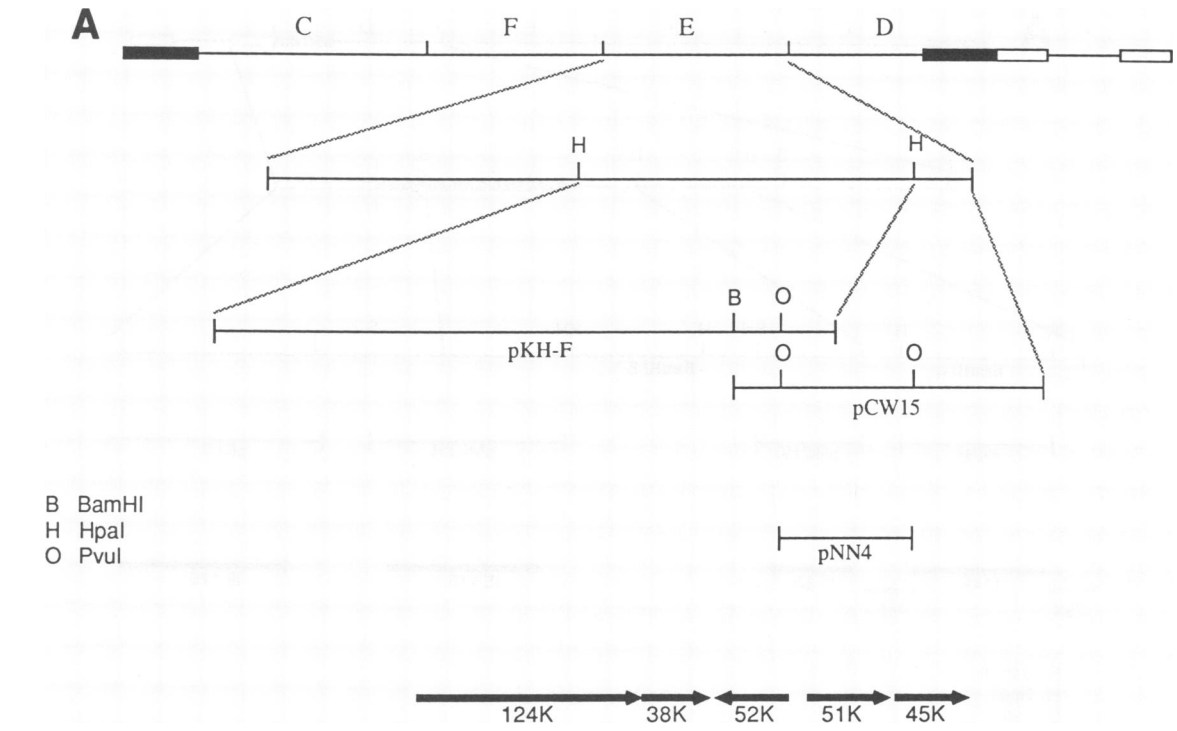
***Xba*I-E.** The genes encoding the two subunits of ribonucleotide reductase are located within *Xba*I-E (40). Since a temperature-sensitive mutation mapping within RR has been reported to cause a defect in the ability to synthesize viral DNA at the restrictive temperature (40), we began our analysis of *Xba*I-E by testing to see whether RR is one of the functions required for plasmid DNA replication. The plasmid pKH-F (*Hpa*I-F) contains both subunits of RR (as well several other genes). pKH-F could not substitute for *Xba*I-E in a standard replication assay (Fig. 2B, lane 5). To locate the gene(s) missing in pKH-F, we constructed plasmids containing the *Xba*I-E sequences on each side of pKH-F. The combination of pKH-F and pCW15, which contained the sequences n *Xba*I-E to the right of the pKH-F site, completely reconstituted origin-dependent DNA synthesis in assay mixtures lacking *Xba*I-E (Fig. 2B, lane 4). Moreover, pCW15 by itself was capable of supporting DNA replication at a somewhat reduced level (Fig. 2B, lane 4). These data suggest that at least one essential gene must be located within pCW15 and that RR is not absolutely required for plasmid DNA replication.

DNA sequence analysis revealed the presence of two intact genes within pCW15 (Fig. 2A). Of these two genes, the one encoding a 51K polypeptide was the only gene required for DNA replication in our assay (Fig. 2C). Plasmid pNN4 contains a fragment which extends from a *Pvu*I site 723 bp upstream of the amino terminus of the 51K open reading frame to another *Pvu*I site 718 bp downstream of its carboxy terminus. pNN4 could substitute for *Xba*I-E in a standard replication assay (Fig. 2C, lane 3), although, as in the case of pCW15, the level of replication observed with pNN4 was somewhat reduced from that seen with *Xba*I-E or with a combination of pNN4 and pKH-F (data not shown). Therefore, *Xba*I-E contains one gene that is essential for plasmid DNA replication and at least one stimulatory gene which we have not located precisely. It seems likely that the stimulatory gene(s) corresponds to one or both of the two subunits of RR.

***Xba*I-D.** The restriction fragment *Xba*I-D contains sequences from the right end of U_L as well as the entire long repeat region. We initially concentrated on finding replication functions within the U_L sequences of *Xba*I-D by including *Eco*RI-JK, which also contains the long repeat region, in our standard assay. Preliminary experiments showed that under such conditions, digestion of *Xba*I-D with *Bam*HI did not reduce its ability to support origin-dependent DNA replication. We therefore cloned three of the fragments produced by *Bam*HI and tested them for their ability to substitute for *Xba*I-D in a standard replication assay. Two of these cloned fragments, *Bam*HI-L and *Bam*HI-B together supported DNA replication at a level equivalent to that seen with intact *Xba*I-D (Fig. 3B, lane 3). No DNA replication was seen with *Bam*HI-B alone (lane 5), but *Bam*HI-L alone supported replication at a reduced level (lane 4). We conclude that there must be at least one gene that is essential for plasmid DNA replication within *Bam*HI-L and one or more stimulatory genes within *Bam*HI-B.

An examination of the sequence of *Bam*HI-L revealed that this fragment contains three intact genes. Deletion analysis (data not shown) of *Bam*HI-L indicated that of these three genes, only the gene encoding a 114K polypeptide was required for plasmid DNA replication. This was confirmed by the data shown in Fig. 3C. Plasmid pNN5 contains a fragment which extends from an *Hpa*I site 164 bp upstream of the amino terminus of the 114K open reading frame to another *Hpa*I site 394 bp downstream of its carboxy terminus. Since pNN5 supported DNA replication as well as *Bam*HI-L did (Fig. 3C, lanes 1 and 3), we conclude that the gene encoding the 114K open reading frame is the only gene within *Xba*I-D that is essential for plasmid DNA replication. DNA sequence analysis suggested that the mRNA encoding the 114K open reading frame was the upstream member of a 3′ coterminal family of mRNAs. Thus, as was the case with the 99K open reading frame in *Xba*I-C described earlier, the 114K protein encoded within plasmid pNN5 must be expressed at a level sufficient for DNA replication in the absence of an obvious polyadenylation site.

As indicated above, our data suggested that *Bam*HI-B contained one or more genes which were not absolutely required for but stimulated plasmid DNA replication in our assay. One (among several) of the genes contained within *Bam*HI-B encodes the immediate-early protein IE63 (1, 29) (Fig. 3A). We suspected that this gene might encode the stimulatory factor in *Bam*HI-B, since it is known that the immediate-early genes IE110 and IE175 stimulate the expression of HSV early genes in transient cotransfection experiments (4, 18, 19, 35, 42), and there is evidence that IE63 has a similar activity on certain HSV-1 genes (19; D. Knipe, personal communication). We decided therefore to test the effect of combinations of all three immediate-early genes normally present in standard replication assays. Accordingly, we constructed two subclones of *Xba*I-D, pMC150 and pMC151, which contain only the gene encoding IE63 or IE110, respectively. Figure 4 shows the results of an



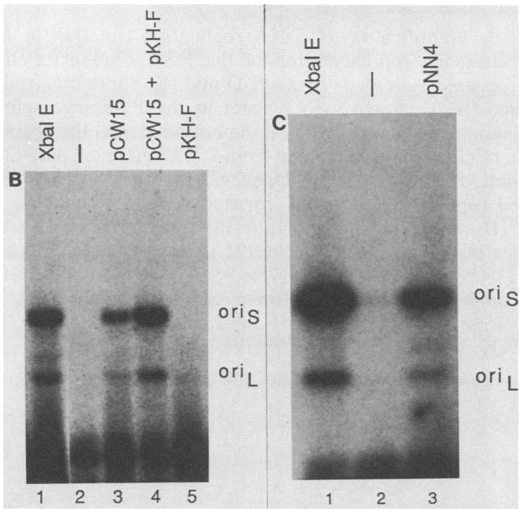


FIG. 2. Identification of DNA replication functions in *Xba*I-E. (A) Diagramatic representation of the HSV sequences contained within plasmid subclones of *Xba*I-E, shown with respect to the P arrangement of the HSV genome. The bold arrows at the bottom depict the arrangement of open reading frames deduced from DNA sequence data (30; D. J. McGeoch, unpublished data). Open and solid bars indicate the short inverted repeat and the long inverted repeat sequences, respectively. (B and C) DNA replication assays. Vero cells were transfected with 0.5 μg each of pMC121 (*Xba*I-C), pMC122 (*Xba*I-F), pMC124 (*Xba*I-D), pK1-2 (IE175), pSG25, and the plasmid(s) indicated at the top of each lane. ori_S and ori_L refer to the positions of *Eco*RI-*Hin*dIII-digested pSG25 and pMC122, respectively, which were run as markers on the same gel.

experiment in which DNA replication was assayed by using mixtures containing *Xba*I-C, *Xba*I-F, *Xba*I-E, *Bam*HI-L, and combinations of the three immediate-early genes. In the absence of any of the three immediate-early genes, no DNA replication was observed (lanes 2 and 10). pK1-2 (IE175) alone was sufficient to stimulate low levels of DNA replication (lanes 3 and 11). Pairwise combinations of pK1-2 (IE175) and pMC150 (IE110) (lanes 6 and 14) or pK1-2 and PMC151 (IE63) (lanes 8 and 16) gave rise to an intermediate level of DNA replication, and a combination of all three immediate-early genes supported DNA replication at a level equivalent to that seen in our standard assay (lanes 1, 9, and 17). It seems likely, therefore, that the stimulatory factor contained in *Bam*HI-B must be the gene which encodes the immediate-early protein IE63.

The data displayed in Fig. 4 also suggested that, as was the case with IE63, neither IE175 nor IE110 was absolutely required for origin-dependent replication in our system. As indicated above, in the absence of IE110, intermediate levels of DNA replication were observed. In the absence of IE175, the combination of genes encoding IE110 and IE63 supported low levels of DNA synthesis (Fig. 4, lane 15), and this low level was increased by the presence of a plasmid containing the gene for the 65K virion *trans*-induction factor (2, 6) (Fig. 4, lane 7). The *trans*-induction factor is known to increase the level of expression of immediate-early genes in transient assays (6). We conclude that while optimal levels of DNA replication are achieved by the presence of the three immediate-early proteins IE175, IE110, and IE63, no single one of these gene products is absolutely required. The significance of these findings will be discussed below.

Seven essential genes and three immediate-early genes are sufficient for plasmid DNA replication. The plasmid complementation analyses presented above indicated that seven

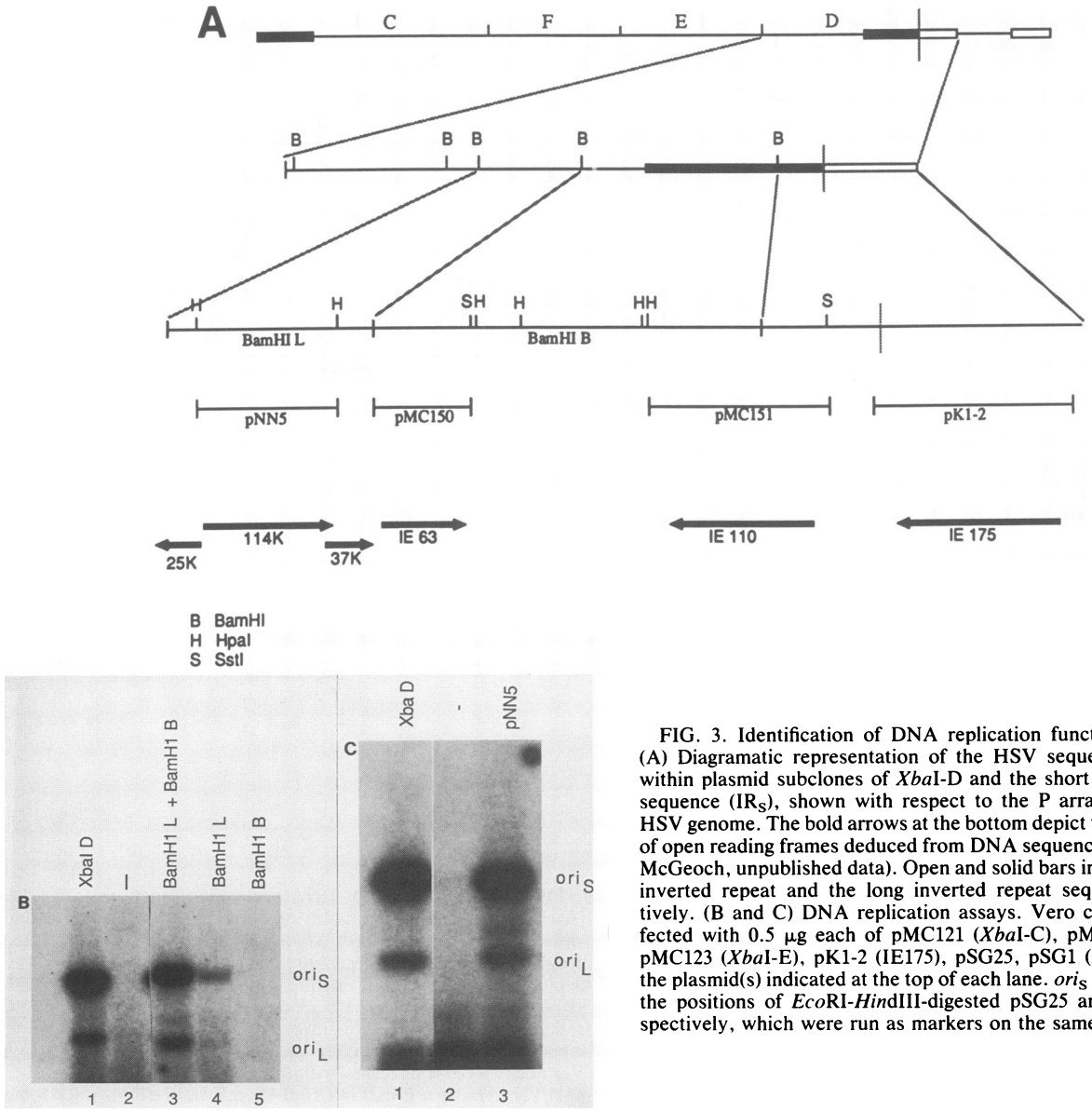


FIG. 3. Identification of DNA replication function in *Xba*I-D. (A) Diagramatic representation of the HSV sequences contained within plasmid subclones of *Xba*I-D and the short inverted repeat sequence (IR_S), shown with respect to the P arrangement of the HSV genome. The bold arrows at the bottom depict the arrangement of open reading frames deduced from DNA sequence data (30; D. J. McGeoch, unpublished data). Open and solid bars indicate the short inverted repeat and the long inverted repeat sequences, respectively. (B and C) DNA replication assays. Vero cells were transfected with 0.5 μg each of pMC121 (*Xba*I-C), pMC122 (*Xba*I-F), pMC123 (*Xba*I-E), pK1-2 (IE175), pSG25, pSG1 (*Eco*RI-JK), and the plasmid(s) indicated at the top of each lane. ori_S and ori_L refer to the positions of *Eco*RI-*Hin*dIII-digested pSG25 and pMC122, respectively, which were run as markers on the same gel.

HSV-1 genes were necessary for origin-dependent DNA replication. The results presented in Fig. 5 show that these seven genes, together with three immediate-early genes, were also sufficient to support plasmid replication. A mixture of 10 plasmids, each containing one of the seven essential genes or one of the immediate-early genes, was cotransfected into Vero cells together with pKB-X, which contains ori_S but no intact HSV gene. This combination of plasmids supported the replication of pKB-X at a level only slightly lower than that seen with the standard assay mixture of four cloned *Xba*I fragments and pK1-2. We conclude that the HSV-1 genome contains no other genes that are essential for origin-dependent plasmid replication. The location and predicted size of the proteins encoded by each of the essential genes are summarized in Fig. 6.

DISCUSSION

In this work we have used a transient complementation assay to locate HSV sequences which encode functions

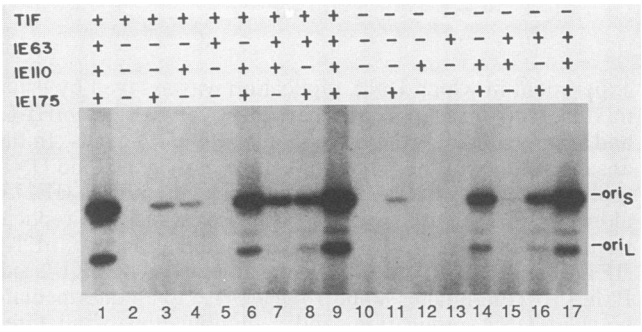


FIG. 4. Effect of immediate-early genes on DNA replication. Vero cells were transfected with 0.5 μg each of pMC121, pMC122, pMC123, pKB-L (*Bam*HI-L), and the indicated combinations of pK1-2 (IE175), pMC150 (IE63), pMC151 (IE110), and pKB-F (*Bam*HI-F, which was used to supply the gene encoding the 65K *trans*-induction factor [TIF]). ori_S and ori_L refer to the positions of *Eco*RI-*Hin*dIII-digested pSG25 and pMC122, respectively, which were run as markers on the same gel.

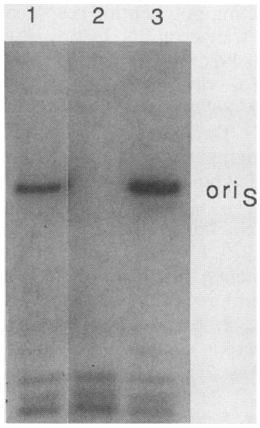


FIG. 5. DNA replication supported by a combination of the seven essential replication genes and three immediate-early genes. Lane 1, Vero cells were transfected with 0.5 μg each of pCW21, pMC160-1, pMC160-2, pNN1, pNN3, pNN4, pNN5, pK1-2, pMC150, pMC151, and pKB-X. Lane 2, Identical to lane 1, except lacking pNN3 as a negative control. Lane 3, Standard replication assay with *Xba*I clones.

involved in origin-dependent DNA replication. Although interpretation of the results presented in this paper depends heavily on the arrangement of viral genes deduced from DNA sequence analysis of selected regions of the HSV-1 genome presented in the accompanying paper (30), all of our results, including deletion analyses of some of the subclones described above (M. D. Challberg, unpublished data), are consistent with the interpretation of the sequence data. In the course of this analysis, we found seven genes without which there was no detectable DNA replication and at least four genes which stimulated DNA replication. Although the distinction between these two classes of genes rests on the sensitivity of our assay and is therefore somewhat arbitrary, we propose that this distinction in fact reflects a fundamental difference in the role that these genes play in viral DNA replication. This proposal is based on the function of those genes about which we have some information. Three of the stimulatory genes were those encoding the immediate-early proteins IE175, IE110, and IE63. Several studies have shown that expression of IE175 is required for early gene expression in HSV-infected cells (16, 37), and all three of these genes are known to stimulate the expression of one or more HSV genes in transient cotransfection experiments (14, 18, 19, 35, 42). Thus, it seems likely that the immediately-early genes function indirectly in the transient replication assay by stimulating the expression of other genes whose products are directly required for DNA synthesis. Conversely, two of the genes which are essential for plasmid replication in our assay encode Pol and DBP. There is ample genetic and biochemical evidence to support the view that these proteins function directly in viral DNA replication (8, 11, 13, 20, 24, 52). By extension, we therefore propose that the four (or more) genes which stimulate plasmid replication encode proteins which act only indirectly in DNA synthesis and that the seven genes which are required for origin-dependent plasmid replication encode proteins which are directly involved in DNA replication.

The relationship between origin-dependent plasmid DNA replication and viral DNA replication *per se* is not known. As mentioned, the genes encoding DBP and Pol are clearly required both for transient plasmid replication and for viral DNA replication. Moreover, mutations affecting DNA replication have been mapped to locations corresponding closely to the locations of four of the other five essential genes that we have identified (Fig. 6). On the other hand, there are other virus mutants with lesions affecting DNA replication which map within genes that are not required for transient plasmid DNA replication. These include mutants with lesions in IE175 (16, 37), IE110 (44, 49), RR (40), and the alkaline exonuclease (33). It is therefore possible that origin-dependent plasmid replication and viral DNA replica-

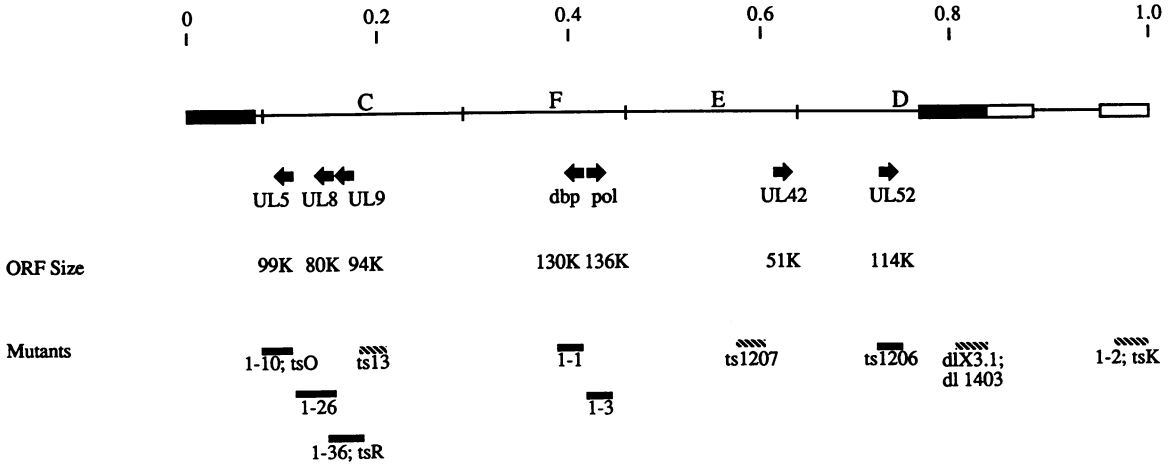


FIG. 6. Summary of essential plasmid replication functions and DNA-negative mutants. The predicted open reading frame (ORF) size and nomenclature for the five essential replication genes with no known function are derived from the DNA sequence analysis of U_L (D. J. McGeoch, manuscript in preparation). Bars denote the locations of mutations in viruses with DNA-negative pheonotypes. Solid bars indicate mutants with lesions in genes which correspond closely to one of the essential plasmid replication functions; hatched bars indicate mutants with lesions in genes which clearly are not essential for plasmid DNA replication. For simplicity, mutations which mapped to the inverted repeat sequences are indicated only once. The data on mutants were compiled from the following sources: complementation group 1-10 (51; S. K. Weller, personal communication); *ts*O, (29) complementation group 1-26 (9; S. K. Weller, personal communication); complementation group 1-36 (S. K. Weller, personal communication); *ts*R (29); *ts*13 (32); complementation group 1-1 (52); complementation group 1-3 (11); *ts*1207 (40); *ts*1206, (29); complementation group 1-2 (16); *ts*K (37); *dl*X3.1 (44); *dl*1403 (49).

tion take place by different mechanisms which happen to involve some of the same gene products. However, the limited information available about the functions of these various genes is consistent with the idea that the seven genes essential for plasmid replication constitute a set of genes whose products are directly involved in viral DNA synthesis, while other genes which affect viral DNA replication but have little or no effect on plasmid DNA replication encode functions which play an auxilliary role in DNA synthesis. Clearly, confirmation of this hypothesis awaits the biochemical characterization of all of these gene products.

The functional significance of the fact that the HSV genome contains three replication origins consisting of two distinct DNA sequences is not known. Studies with mutant viruses have shown that deletion of ori_L or one copy of ori_S has little or no effect on the replication of the virus in cultured cells (26, 36a). This, together with the fact that the nucleotide sequences of ori_S and ori_L have extensive homology (43, 53), suggests that ori_S and ori_L are functionally equivalent. The data reported in this paper lend further support to this view: we found no viral gene products which had a specific effect on either ori_L- or ori_S-dependent plasmid replication. It remains possible that even though the same viral gene products participate in initiating replication at the two origins, the mechanism of initiation differs at the two sites, or that one of these two origins is specifically involved in some aspect of viral DNA replication which is not reflected in the transient assay system. Additional work will be required to address these possibilities.

In summary, we have identified a set of HSV-1 genes which are both necessary and sufficient to support origin-dependent DNA replication. The function of only two of the products of this set of seven genes is known, namely the DNA Pol and the single-stranded DBP. By analogy with well-characterized procaryotic replication systems, we can suggest functions for the remaining five genes. Such functions might include a primase, a helicase, a topoisomerase, accessory factors which increase the processivity or efficiency of the DNA Pol, and a protein which specifically binds to the HSV origins of DNA replication. Identification of this complete set of genes and determination of their sequences should facilitate the isolation of and assignment of specific biochemical function to each HSV protein involved in DNA synthesis.

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