The adenovirus terminal protein precursor functions as a primer for the initiation of virus DNA replication by covalently binding the first nucleotide in the DNA chain. It remains covalently attached to the 5'-ends of the virus DNA and is cleaved to the terminal protein during virion maturation. The gene encoding the terminal protein precursor maps within a 7-kilobase region of the virus genome, which specifies multiple mRNA and protein species. We have determined the location, within this region, of the coding sequences for the terminal protein precursor by aligning partial amino acid sequence to the amino acid sequence predicted from the DNA sequence (accompanying papers; Gingeras, T. R., Sciaky, D., Gelinas, R. E., Bing-Dong, J., Yen, C. F., Kelly, M. M., Bullock, P. A., Parsons, B. L., O'Neill, K. E., and Roberts, R. J. (1982) J. Biol. Chem. 257, 13475–13491; Aleström, P., Akusjarvi, G., Pettersson, M., and Pettersson, U. (1982) J. Biol. Chem. 257, 13492–13498). The open translational reading frame between coordinates 23.4 and 28.9 on the genome contains the majority of the coding sequences for the precursor protein. The virion terminal protein derives from COOH terminus of the precursor protein. Using this information, the site within the protein of covalent attachment to the DNA has been determined. This site also corresponds to that which covalently binds dCMP; the first nucleotide in nascent DNA synthesized in vitro. The coding region for terminal protein precursor does not overlap the region to which the N-group of adenovirus mutants has been mapped. We suggest that these mutants define a protein, other than terminal protein, which also functions in the initiation of virus DNA replication.

A novel mechanism for the initiation of adenovirus DNA replication, originally proposed by Bellett and co-workers (Robinson and Bellett, 1974; Rekosh et al., 1977), involves the priming of DNA chain elongation by a deoxynucleotide covalently attached to a protein. This mechanism has recently found wide support (Challberg et al., 1980; Lichy et al., 1981; Enomoto et al., 1981; Challberg et al., 1982; Tamanoi and Stillman, 1982) and the protein has been identified as pTP.\footnote{Present address, Biogen Inc., 241 Binney St., Cambridge, MA. 02142.}

\footnote{The abbreviations used are: pTP, precursor to the terminal protein; 140K protein, protein of $M_\text{r} = 140,000$ (other forms expressed similarly); ad2, adenovirus type 2; E2B, early transcription unit; SDS, sodium dodecyl sulfate.}

This protein is covalently attached to the termini of replicating DNA in vivo (Coombs et al., 1978; Kelly and Lechner, 1978; Stillman and Bellett, 1978, 1979; Van Wielink et al., 1979; Challberg and Kelly, 1981) and nascent DNA synthesized in vitro (Challberg et al., 1980; Stillman, 1981). The unbound form of pTP can covalently bind dCTP to form a pTP-dCMP complex in a reaction which requires specific DNA sequences at the origin of replication (Lichy et al., 1981; Pincus et al., 1981; Tamanoi and Stillman, 1982; Challberg et al., 1982). Recently, Enomoto et al. (1981) have purified functional pTP and an associated 140K protein and demonstrated that these proteins together contained the dCMP binding activity and a DNA polymerase activity.

The mature virion TP was first recognized as enabling virus DNA to circularize via a protease-sensitive, noncovalent interaction (Robinson et al., 1973; Robinson and Bellett, 1974) and subsequently identified as a 55K protein covalently linked to each 5'-end of the linear virus DNA (Rekosh et al., 1977). The covalent linkage between DNA and protein is a phosphodiester bond between the b-hydroxyl group of a serine residue in the protein and the 5'-hydroxyl of the terminal deoxycytosine residue (Desiderio and Kelly, 1980). That TP is derived from a precursor protein was first suggested by the detection of an 80,000-dalton protein that was covalently linked to the 5'-end of nascent DNA replicated in cell-free extracts prepared from virus-infected cells (Challberg et al., 1980). The 80K protein is structurally related to TP and is also covalently linked to the DNA by the same phosphodiester bond.

The origin of TP had long been an enigma until the demonstration by cell-free translation of hybridization-selected mRNA that proteins with estimated $M_\text{r} = 105,000, 87,000$, and 75,000 are encoded by the virus l-strand between coordinates 11 and 31.5 and that the 87K protein is structurally related to TP (Stillman et al., 1981). The 87K protein is the same as the 80K protein described by Challberg et al. (1980); the discrepancy is due to the use of different molecular weight markers. In addition, the 87K protein is structurally related to the 80K–87K protein that is covalently associated with the DNA from ad2 ts1 virions grown at the nonpermissive temperature (Stillman et al., 1981; Challberg and Kelly, 1981), a mutant virus that fails to cleave virus-encoded precursor proteins to their mature counterparts during virion maturation (Bhatti and Weber, 1979). The mapping of pTP to the virus genome also led to the definition of a new early transcription unit, designated E2B. Several mRNAs from E2B have been identified, including those containing leaders at coordinates 39, 68.5, and 75 linked to various RNA main bodies, which extend from either coordinate 30, 25, or 23 to coordinate 11.1 (Stillman et al., 1981). The existence of multiple mRNA and protein species and the length of the E2B region of the virus genome (approximately 7000 base pairs) are such that the location of the coding region for pTP was not known. Recently, Gingeras et al. (1982) and Aleström et al. (1982) have deter-
determined the DNA sequence of this region of the Ad2 genome and found that it contains three large open translational reading frames, two of which could encode pTP. To determine the region of DNA encoding pTP, we have determined the partial sequence of radiolabeled peptides and aligned these to the amino acid sequence predicted from the DNA sequence. Furthermore, we have been able to define a functional site within pTP, namely the site of covalent linkage of virus DNA. This data, together with the DNA sequence data (Gingeras et al., 1982; Alestrom et al., 1982), and the mapping of a group of DNA negative mutants in this region (Galos et al., 1979) suggest that an additional virus-coded protein from the E2B region functions in DNA replication.

**EXPERIMENTAL PROCEDURES**

**Cells and Viruses**—Wild type adenovirus type 2 was grown in suspension cultures of HeLa cells and ad2 ts1, provided by J. Weber, University of Sherbrooke, Quebec, was grown in monolayers of HeLa cells at either 32 °C (permissive temperature) or 40 °C (nonpermissive temperature). The viruses were purified as described previously (Loneberg-Holm and Philipson, 1969).

**Purification of DNA-Protein Complexes**—DNA-protein complexes from ad2 wild type or ad2 ts1 virions were purified as described previously (Stillman et al., 1981). For labeling the protein with [35S]methionine by autoradiography of dried gels, excised, oxidized with performic acid, and digested with N-tosylphenylalanine chloromethyl ketone-trypsin while still in the gel slice, as described by Smart and Ito (1978).

**Chromatography of peptides through a Spherisorb ODS (C-18) reverse phase column (Spectra-Physics) on a Spectra-Physics SP8000 high performance liquid chromatography system was as described by Smart et al. (1981). Similarly, peptide sequence analysis using the spinning cup method on a Beckman 890C sequencer has been described previously (Smart et al., 1981).

**RESULTS**

**Isolation of Radiolabeled Proteins**—To obtain partial protein sequence of pTP and its related proteins, we radiolabeled the protein by three independent methods. First, we labeled only pTP with [35S]methionine by translation of selected mRNA in a rabbit reticulocyte lysate. E2B-specific RNA was selected by hybridization and after cell-free translation, the products were separated by electrophoresis in a preparative gel. An analytical gel of approximately 2% of the product is shown in Fig. 1A and the 87K pTP is indicated. Other proteins

![Fig. 1. SDS-polyacrylamide gel electrophoresis of radiolabeled pTP and related proteins. A, [35S]methionine-labeled proteins obtained by translation of E2B-selected mRNA in a rabbit reticulocyte lysate. B, [35S]methionine-labeled proteins that were covalently linked to the DNA from ad2 ts1 virions grown at 32 °C. C, same as B, except that protein was covalently linked to the DNA from ad2 ts1 virions grown at 40 °C. D, [35S]methionine-labeled E2B-specific proteins, 2 mg of total cytoplasmic RNA were used for selections and the selected mRNA was translated in a 200-μl reaction mixture that contained 800 μCi of [35S]methionine (New England Nuclear) and 80 μl of a rabbit reticulocyte lysate. The products were separated on preparative 10% SDS-polyacrylamide gels as described by Laemmli (1970).](image_url)
selected by this plasmid DNA have been discussed previously (Stillman et al., 1981).

The second source of radiolabeled protein was obtained by iodination of proteins covalently attached to the virion DNA from ad2 tsl which was grown at either 32 °C (permissive temperature) or 40 °C (nonpermissive temperature). The iodinations, by the chloramine-T method, were performed with and without extensive denaturation of the protein. Proteins were removed from the DNA by treatment with either deoxyribonuclease I or piperidine and separated by gel electrophoresis (Stillman et al., 1981; Fig. 1, B and C). At the nonpermissive temperature, only the precursor 87K protein was detected (Fig. 1C). However, at the permissive temperature, three proteins of M, = 87,000, 62,000, and 55,000 were detected (Fig. 1B).

The third method for radiolabeling pTP was by the formation of a 32P-labeled pTP-dCMP complex in a cell-free extract prepared from adenovirus-infected HeLa cells. DNA-protein complex, the template for replication in vitro, and [α-32P]dCTP were incubated with the DEAE-cellulose fraction (see "Experimental Procedures") and the reaction mixture was then subjected to electrophoresis in a preparative gel. An analytical gel of approximately 4% of the reaction mixture is shown in Fig. 1D and reveals a single labeled band at the position of the pTP and contains pTP covalently linked to a dCMP residue (Lichy et al., 1981; Challberg et al., 1982).

Tryptic Peptide Maps of Radiolabeled Proteins—The [3S]methionine-labeled 87K protein that is translated from E2B-selected mRNA has been shown to be structurally related to both the 35S-labeled 87K pTP and 55K pTP that are covalently attached to the DNA of ad2 tsl virus grown at 40 °C and wild type ad2 virus respectively (Stillman et al., 1981). For reference, Fig. 2 shows a tryptic peptide map of [35S]methionine-labeled 87K pTP that was obtained by translating mRNA preparatively selected by the d2 Ball-E fragment. Because very few counts were obtained by labeling pTP-related proteins with [3S]methionine in vivo, we demonstrated that the 62K protein that is covalently bound to the DNA of ad2 tsl virus grown at 32 °C was related to pTP and TP by labeling these proteins with 32P (Fig. 1, B and C). Tryptic peptide maps of the 97K pTP from ad2 tsl virus grown at 40 °C and the 87K pTP, 62K, and 55K pTP proteins from ad2 tsl virus grown at 32 °C are shown in Fig. 3. The labeled proteins were removed from the DNA by treatment with either DNase I or with piperidine before preparative gel electrophoresis and digestion with trypsin.

![Fig. 2. Reverse phase (C-18) chromatography of the [3S]methionine-labeled tryptic peptides of pTP obtained from translation of E2B hybridization-selected mRNA in a rabbit reticulocyte lysate. 280 µl of each 700-µl fraction were taken for counting and the highest peak (C) contains 2000 cpm. Each peak that was pooled for automatic sequence analysis was allocated a letter (A–O).](image)
FIG. 3. Reverse phase (C-18) chromatography of the $^{125}$I-labeled tryptic peptides of 87K pTP, 62K, and 55K TP obtained from ad2 ts1 virus grown at either 32 or 40 °C. Left, proteins were removed from the DNA by digestion with DNA I. Right, proteins were removed from the DNA by treatment with piperidine. 280 µl of each 700-µl fraction were taken for counting and the highest peak contained: 87K-40 °C DNase, 17,465 cpm; 87K-32 °C DNase, 12,384 cpm; 62K-32 °C DNase, 9,755 cpm; 55K-32 °C DNase, 6,724 cpm; 87K-40 °C piperidine, 27,438 cpm; 87K-32 °C piperidine, 28,622 cpm; 62K-32 °C piperidine, 4,719 cpm; 55K-32 °C piperidine, 6,827 cpm. Each peak that was pooled for automatic sequence analysis was allocated a letter (A-J).

FIG. 4. Automatic sequential Edman degradation of [35S]methionine-labeled tryptic peptides of pTP. Peptides A-O (Fig. 2) were sequenced and the percentage of total counts/min applied is shown for each residue number. Each line on the vertical axis represents 5% of the total counts/min applied and the per cent counts/min remaining in the spinning cup is shown as a bar on the right. Total counts/min applied were: A, 1854; B, 4068; C, 4796; D, 2440; E, 2506; F, 1820; G, 3978; H, 2978; I, 4824; J, 2936; K, 5282; L, 3328; M, 2790; N, 2796; O, 2384.
and 24.3. These peptides contain methionines at positions 2 (either G, H, I, K, or M) and 15 (either M or O) and may derive from coding sequences not present in this region (see “Discussion”). However, this analysis identifies the open reading frame in the DNA sequence between coordinates 22.9 and 23.4 as that which encodes at least the majority of pTP.

The low number of counts obtained by \(^{35}\)S-labeling of pTP and its related proteins in vivo made it impractical to do partial amino acid sequence analysis on \(^{35}\)S-labeled pTP-related proteins and both contain a tyrosine at position 55K. These peptides containing tyrosines cause peptides to chromatograph as two peaks on the reverse phase columns and the fact that only a fraction of the predicted tyrosines were easily labeled by this procedure.

Peak J, and the related peptide J\(_2\) (Fig. 3) are present in all pTP-related proteins and both contain a tyrosine at position 12 (Fig. 5). A peptide with a tyrosine at position 12 is unique in the predicted amino acid sequence (Fig. 7) and is in the COOH terminus half of the molecule. This peptide also contains a methionine at position 15 and is either peptide M or O (Fig. 4). Similarly, peak I and its related peptide eluting in fractions 138-145 are present in all three proteins, but only when the protein is removed from the DNA by cleavage with piperidine. Peptide I contains a tyrosine at position 17 (Fig. 5); only two such peptides are present in the predicted amino acid sequence and both are near the COOH terminus of the protein. We have identified peptide I as that which is covalently linked to the DNA (see below). Peptide D, which contains a tyrosine at position 8 (Fig. 5), is also present in all three proteins (Fig. 3) and corresponds to a unique predicted peptide that also lies near the COOH terminus of the protein. Peptide H, which is only observed in the 55K protein, contains a tyrosine at position 2 (Fig. 5). Although two such peptides are predicted in the amino acid sequence, one lies within the COOH terminus half of the pTP (Fig. 7, line 8). This tyrosine is probably made accessible to iodination after amino acids are removed during the maturation of 87K to 55K. This identifies the COOH-terminal end of pTP as that part of the protein from which the 62K and 55K proteins originate.

We have also identified peptides that are present in the 87K and 62K proteins, but absent from the 55K protein. Peptides B, C, or B/C (Fig. 3) contain tyrosines at positions 5, 6, and 5 and 6, respectively (Fig. 5). Similarly, peptides G and F contain tyrosines at positions 5 and 6 (Fig. 5) and may be the diiodinated counterparts to peptides B, C, or B/C. These peptides are only present in the 87K and 62K proteins, but not the 55K protein (Fig. 3). Peptides containing tyrosines at positions 5 and 6 are toward the NH\(_2\) terminus of the amino acid sequence of pTP, again indicating that the 55K protein is completely contained within COOH terminus of the pTP protein.

Finally, we have identified two peptides that are only present in the precursor 87K protein. Peptides A and E (Fig. 3) contain tyrosines at positions 2 and 1, respectively, and are only present in the 87K protein. Two predicted peptides contain a tyrosine at position 1 and both are near the NH\(_2\) terminus of the amino acid sequence. Also in this region is a short tryptic peptide that contains a tyrosine at position 2 and is most likely to correspond to peptide A because of its early elution from the reverse phase column. Thus, sequence analysis of iodinated peptides has determined the relative positions within the predicted amino acid sequence of pTP, and of the 62K and 55K proteins. These are indicated in Fig. 8.

**Identification of the Site of Covalent Attachment of Virus DNA to pTP—DNA-protein complex prepared from ad2 ts1 virions was denatured and then iodinated by the chloramine-T method. The DNA-protein complex was then digested with either trypsin or V8 protease and the DNA then separated from free peptides by equilibrium centrifugation in CsCl gradients containing 4 M guanidine HC1. The peptides that remained bound to the DNA were labeled with \(^{125}\)I, which indicated that a tyrosine was near the DNA attachment site. They were removed by treatment with piperidine and then subjected to reverse phase chromatography (Fig. 6, A and B). The tryptic peptide eluted as a doublet (Fig. 6A) with approximately the same retention time as the peptide that was only released from the DNA by treatment with piperidine (Fig. 3). The peak eluting in fractions 139-145 (Fig. 6A) contained a peptide with the \(^{125}\)I label at position 17 (Fig. 6E). The other peak in Fig. 6A corresponded to peak I (Fig. 3) which also contained a tyrosine at position 17 (Fig. 5). Similarly, the peptide released by digestion with V8 protease eluted as a doublet and the first peak contained a peptide with the \(^{125}\)I label at position 3 (Fig. 6F). This revealed the identity of the peptide that is covalently attached to the virus DNA as a unique peptide in the predicted amino acid sequence (Fig. 7). This peptide also contained a methionine at position 12, which corresponds to peptide L (Figs. 2 and 4, line 8). This tyrosine is probably made accessible to iodination after amino acids are removed during the maturation of 87K to 55K. This identifies the COOH-terminal end of pTP as that part of the protein from which the 62K and 55K proteins originate.

| TABLE I |
|-----------------|-----------------|-----------------|-----------------|
| Position of methionine residues in tryptic peptides of pTP | Number of peptides predicted | Peptides observed |
| Position of methionine | 22.9-14.2 | 28.9-23.4 | 22.9-14.2 | 28.9-23.4 |
| 1 | 1 | 1 | 1 (B) |
| 2 | 3 | 4 | 5 (G,H,K,L) |
| 3 | 1 | 2 | 2 (D,F) |
| 4 | 0 | 1 | 2 (N,E) |
| 5 | 0 | 1 | 0 |
| 6 | 2 | 0 | 0 |
| 7 | 0 | 1 | 1 (F) |
| 8 | 1 | 1 | 1 (J) |
| 9 | 2 | 0 | 0 |
| 10 | 1 | 3 | 2 (J,K) |
| 11 | 0 | 1 | 1 (L) |
| 12 | 0 | 1 | 0 |
| 13 | 0 | 1 | 1 |
| 14 | 0 | 1 | 2 (M,O) |
| 15 | 0 | 1 | 0 |
| 16 | 1 | 1 | 1 |
| 17 | 0 | 1 | 0 |
| 18 | 0 | 1 | 1 (M,N,O) |
| 20 | 0 | 1 | 0 |
| 21 | 0 | 1 | 0 |
| 22 | 0 | 1 | 0 |

- The position of a methionine in the amino acid sequence following a trypsin cleavage site.
- The number of methionine-containing tryptic peptides in the amino acid sequence derived from the DNA sequence between coordinates 22.9 and 14.2.
- The same d as for coordinates 28.9-23.4.
- The number of peptides obtained by automatic Edman degradation after tryptic digestion of \(^{125}\)Smethionine-labeled pTP. The data are a summary of data presented in Fig. 4 and the identity of each peptide is indicated in parentheses. These data do not include peak A (Fig. 3) which was obtained in variable amounts between experiments.
- These peptides contain two methionines at positions 5 and 7, 6 and 9, and 11 and 20, respectively.
labeled peptides that were covalently attached to the DNA; the slight difference in elution is probably due to the remaining dCMP residue attached to the peptides, which is more evident for the shorter V8 protease peptide. This suggested that the DNA attachment site and the dCMP binding site were one and the same. Attempts, by automatic sequence analysis, to determine the position within each peptide of the serine residue that is linked to the dCMP residue via a phosphodiester bond (Challberg et al., 1980; Lichy et al., 1981; Challberg et al., 1982) were not successful, again probably due to the dCMP residue attached to the peptide. However, each peptide only contains one serine residue (Fig. 7).
Fig. 7. The amino acid sequence predicted from the DNA sequence for the open translational reading frame on the 1-strand between termination codons at coordinates 28.9 and 23.4. Each line contains 70 amino acids. Numbers above and below each line indicate the position of a methionine or tyrosine, respectively, from the NH₂ terminus of a trypsin cleavage site. The underlined sequence is discussed in the text and broken underlined sequence is homologous to the proteolytic cleavage site utilized for maturation of protein pVI to virion protein VI (Akusjarvi and Persson, 1981). The site of covalent linkage of virus DNA to the protein is indicated (A).

DISCUSSION

The adenovirus terminal protein has been the object of considerable interest because of its novel function in the initiation of virus DNA replication. The precursor form of the protein was shown to be encoded by the early region E2B in the genome (Stillman et al., 1981), which contains a large coding capacity between coordinates 11 and 30. The precursor protein was shown to be structurally related to the virion terminal protein (Challberg et al., 1980; Stillman et al., 1981) and is present in replication extracts from virus-infected cells, on the terminus of ad2 ts1 virion DNA and on the terminus of intracellular replicating virus DNA. A protein of 62,000 daltons is also associated with the virion DNA of the ad2 ts1 mutant and we have demonstrated that it is also structurally related to both TP and pTP. This protein may therefore represent an intermediate cleavage product of pTP produced during maturation of the virion.

Since the mapping of the gene encoding pTP on the virus genome, the DNA sequence of the E2B region has been determined for both ad2 (Gingeras et al., 1982; Alestrom et al., 1982) and ad7. Both DNA sequences show extensive regions of homology in the large open reading frames on the 1-strand DNA. Indeed, Green et al. (1979) and Rekosh (1981) have shown a high degree of conservation between the terminal proteins from a number of adenovirus serotypes. We have obtained partial amino acid sequence of peptides from all three TP-related proteins from ad2 and have aligned these sequences to the amino acid sequence predicted from the DNA sequence. These data were sufficient to enable us to distinguish among the three large open translational reading frames in the E2B region. The gene encoding pTP maps to the right-hand end of early region E2B between coordinates 28.9 and 23.4 (Fig. 8). This open reading frame would produce a 74,709-dalton protein if translation were to begin at the first methionine residue in the sequence (however, see below). Thus, it is most likely that pTP is translated from the largest E2B mRNA that has leaders at coordinates 75, 68, and 39 and a main body between approximate coordinates 30 and 11 (Fig. 8) (Stillman et al., 1981). Both the 62K and 55K proteins derive from the COOH-terminal end of pTP and the exact location of the proteolytic cleavage sites are currently being determined. However, we note that there exists a predicted amino acid sequence within pTP (Asp-Met-Thr-Gly-Gly-Val-Phe; Fig. 7) that has striking similarity to the proteolytic cleavage site utilized for maturation of protein pVI to virion protein VI (Asn-Met-Ser-Gly-Gly-Ala-Phe; Akusjarvi and Persson, 1981). This latter cleavage is also affected by the ad2 ts1 mutation (Weber, 1976). Another region of interest is a sequence that contains repeated amino acids in tandem (Arg-Val-Pro-Glu-Glu-Glu-Ala-Leu-Met-Glu-Ile-Glu; Fig. 7), which has unusual charge and conformational properties. Since this sequence is near the expected NH₂ terminus of the 55K TP, it may separate two functional domains in the precursor protein. This is also a site of DNA sequence heterogeneity between different ad2 DNA molecules (Gingeras et al., 1982).

This study does not define the COOH terminus of pTP due to the lack of methionine and tyrosine residues in this region; however, we have excluded the long open translational reading frame to the left of coordinate 22.3 as a part of pTP. Similarly, the nature of the NH₂ terminus of pTP remains unclear and is not clarified by the DNA sequence of the region between coordinates 30 and 11. Attempts to directly sequence the NH₂ terminus of [³⁵S]methionine-labeled pTP synthesized by translation of selected mRNA have not been successful, which may indicate that the NH₂ terminus is blocked. The first AUG in frame with pTP coding frame occurs 16 codons from the upstream terminator and the amino acids between this terminator codon and the AUG are highly conserved in ad2 and ad7. This terminator codon also corresponds to the point where the ad2 and ad7 DNA sequence homology diverges and also the site where there is a viable, 2-base deletion in ad5 dl309 DNA (Thimmappaya et al., 1979). These factors place a limit upon how far the coding region extends toward the right end of the virus genome (Fig. 8). We consider it likely that a small amount of coding region from the leader at coordinate 39 is juxtaposed by RNA splicing to the long open reading frame discussed here. Indeed, a consensus splice acceptor site overlaps with the terminator codon and the Xbal site at coordinate 28.9 (Gingeras et al., 1982; Alestrom et al., 1982). We are currently making cDNAs across this splice.
proteins were able to complement and that it corresponds to the 140K protein that co-purifies virus DNA. It is most likely that this protein is encoded by the large open reading frame in the genome to which the N-complementation group mutants had attached virus DNA is perfectly conserved between ad2 and ad7, suggesting that this region of the protein plays an important enzymatic role in the initiation of DNA replication.

An unexpected result from the mapping of the coding region for pTP was that it did not overlap with the region of the genome to which the N-complementation group mutants had been mapped by marker rescue experiments (coordinates 18–22.5; Galos et al., 1979). These mutants, including ad5 ts06 and ts149, fail to synthesize virus DNA at the restrictive temperature (Wilkie et al., 1973; Ginsberg et al., 1974; van der Vliet and Sussenbach, 1975) and also fail to transform rodent cells at this temperature (Williams et al., 1979). Thus, a virus-encoded protein, in addition to the 72K single strand DNA binding protein and pTP, must function in the replication of virus DNA. It is most likely that this protein is encoded by the large open reading frame in E2B (coordinates 24–14.2) and that it corresponds to the 140K protein that co-purifies with pTP (Enomoto et al., 1981). The 140K/pTP-purified proteins were able to complement in vitro, inactive extracts prepared from ad5ts149-infected cells.3

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