allow us to make this correlation. First, an altered sequence (ΔIRE) is unable to confer iron-dependent translational control. Second, this altered sequence is incapable of interacting with cytosolic protein as evidenced by its failure either to form a retardation complex or to significantly compete with an IRE in the formation of that complex. This strict correlation of binding and function is analogous to the interaction of the R17 coat protein with an mRNA stem-loop structure in which a single base change in the loop eliminates binding (*14*).

The role of the IRE-binding protein in the function of the IRE was further established by experiments in which intracellular iron levels were manipulated. When K562 cells were treated with the iron-chelator desferrioxamine, the specific activity of the IRE-binding protein was increased four- to fivefold, a finding that is in agreement with the biosynthetic rates of IRE-containing mRNAs in untreated- and chelator-treated cells. Leibold and Munro have demonstrated the loss of an ultraviolet cross-linked protein-ferritin mRNA complex in cells treated with iron (*9*).

We have recently identified a group of IREs within the 3′ UTR of the human transferrin receptor mRNA (*8*). The region containing these IREs was shown to be responsible for iron-dependent regulation of transferrin receptor mRNA levels. We have found that the RNA of this 3′ regulatory region can specifically compete with the 5′ ferritin IRE for the interaction with the IRE-binding protein (*15*). Thus, the IRE-binding protein may be involved in regulation of expression of more than one protein involved in cellular iron metabolism. Many unanswered questions remain concerning the observations reported here. Answers to these questions will require purification of the factor or factors that are responsible for the specific interactions demonstrated in this study.

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10. The 5′ UTR probe was cloned from a Hae III–Eag I fragment representing bases −209 to −124 of ferritin H chain mRNA (*5*) into Hind III–Xma III sites of the pGEM 3-blue (Promega) polylinker after partial fill-in of the gap with the Klenow fragment of DNA polymerase I. The resultant plasmid was linearized with Eco RI and RNA transcripts produced by SP6 polymerase. The IRE probe was cloned from the previously described construct L5 (+26-nucleotide oligomer)-CAT (*7*). A 5′ blunt-ended Bgl I–Xba I fragment was gel-isolated and cloned into the Hind III–Xba I sites after Klenow fill-in of the Hind III site of the plasmid vector pGEM-3 zf(+) (Promega). The same approach was used to generate the ΔIRE probe from the plasmid designated Δ−165 (*7*). Both constructs contain ferritin promoter sequences and the first six bases of the ferritin 5′ UTR in addition to the IREs. Sequences were confirmed by using a modification of the dideoxy chain termination method of sequencing. Riboprobes were generated with SP6 RNA polymerase. β-globin transcripts were generated from a β-globin cDNA cloned into pSP64 [A. R. Krainer, T. Maniatis, B. Ruskin, M. R. Green, *Cell* **36**, 993 (1984)] and were linearized at an Acc I site 150 bases into the cDNA. Riboprobes were generated by the use of SP6 RNA polymerase.
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17. The plasmid designated −IRE corresponds to the L5-CAT plasmid described in (*5*). Transient transfections of plasmids and subsequent determinations of CAT activity were performed as described (*5*).

18. We gratefully acknowledge contributions of J. Casey, D. Koeller, J. Barriocanal, and A. Dancis to this work. S.W.C. was supported by the Dermatology Branch, National Cancer Institute, NIH. We thank E. Perry for her excellent work in preparation of this manuscript.

27 April 1988; accepted 27 June 1988

Transcription Factor OTF-1 Is Functionally Identical to the DNA Replication Factor NF-III

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Octamer transcription factor–1 (OTF-1) and nuclear factor III (NF-III) are sequence-specific DNA binding proteins that activate transcription and DNA replication, respectively. It is shown here that OTF-1 is physically and biologically indistinguishable from NF-III. This conclusion is based on the following observations. First, the two proteins have identical mobilities by SDS–polyacrylamide gel electrophoresis. Second, OTF-1 binds to the adenovirus origin of DNA replication at the same site and with the same affinity as NF-III. Third, OTF-1 can substitute for NF-III in activating the initiation of adenovirus DNA replication in vitro. Fourth, the ability of OTF-1 to stimulate viral DNA replication is dependent on the presence of an intact NF-III binding site within the origin of replication. Fifth, NF-III can substitute for OTF-1 in activating in vitro transcription from the human histone H2b promoter. These data suggest the possibility that NF-III/OTF-1 is a protein that functions in both cellular DNA replication and transcription.

IT IS BECOMING INCREASINGLY APPARENT that the regulation of all nucleic acid synthesis may occur through the function of sequence-specific DNA binding proteins. Transcription promoters are repressed or activated (*1, 2*) and origins of DNA replication become functional (*3*) in the presence of proteins that recognize and bind specific DNA sequences. The identification and characterization of such proteins are the first steps in developing an understanding of how these proteins perform their regulatory role.

Fractionation of the adenovirus DNA replication reaction in vitro (*4*) has allowed the identification of several proteins that can participate in the replication of DNA within virus-infected HeLa cells. Three of these proteins are viral in origin: the 80-kD preterminal protein (pTP), the 140-kD DNA polymerase (Ad Pol), and the 72-kD DNA binding protein (DBP) (*4*). The remaining proteins are cellular in origin: a topoisomerase, NF-II (*5*), and two sequence-specific DNA binding proteins, NF-I (*6, 7*) and NF-III (*8, 9*). We have recently purified NF-III

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and found it to be a single polypeptide of 92 kD (*10*).

Similarly, reconstitution of several different transcription systems in vitro has shown that accurate initiation by RNA polymerase II requires a number of different soluble factors. General transcription factors, such as TFII B, E, and D, are required for the transcription of all class II genes (*11*). In contrast, promoter-specific factors may act on a wide variety of promoters, or they may interact with only a limited set of genes (*2, 12*). Several of these factors have a sequence-specific binding activity, and stimulation of transcription is dependent on binding to the cognate sequence element (*13–15*). One such transcriptionally active, sequence-specific, DNA binding protein, octamer transcription factor-1 (OTF-1), has been purified from HeLa cells (*15*). This protein binds to a DNA sequence (referred to as the octamer sequence) found within the human histone H2b promoter as well as within a number of other promoters and enhancers (*15*). It has been noted that the octamer sequence is remarkably similar to the sequence contained within the adenovirus origin of replication (Ad Ori) recognized by NF-III (*8, 10*). NF-III can recognize the octamer sequence contained within the human histone H2b promoter (*10*). We now present evidence that OTF-1 can recognize the Ad Ori and stimulate DNA replication in vitro in a manner indistinguishable from NF-III.

In previous characterizations of both OTF-1 and NF-III, elution of specific DNA binding activity from SDS-polyacrylamide gels showed that both of these proteins are about 90,000 daltons in size (*10, 15*). To ascertain whether these proteins are of identical size, we subjected preparations of OTF-1 and NF-III to SDS–polyacrylamide gel electrophoresis (PAGE) in adjacent lanes (Fig. 1). Although the NF-III used was from a late step in purification, it was not the only polypeptide species in the sample. It was clear, however, that in both preparations the only polypeptides in the region of 90 kD were of indistinguishable size. Each lane was loaded with an equal quantity of Ad Ori–specific DNA binding activity. Thus the equal staining intensity of the polypeptides suggests that OTF-1 and NF-III also have approximately equal affinities for the Ad Ori. The preparation of OTF-1 analyzed in lane b of Fig. 1 was used in all of the following experiments.

The sequence specificity of OTF-1 binding activity on the Ad Ori was determined by a deoxyribonuclease (DNase) I protection assay. Previous analysis of the DNase I footprint pattern of NF-III on the Ad Ori indicated that this protein protects bases 34 to 52 of the Ad Ori (*9*). OTF-1 protects the same sequence (nucleotides 35 to 52) within the Ad Ori from DNase I (Fig. 2A). Similarly, NF-III protects the octamer sequence within the H2b promoter (nucleotides −36 to −55) (*10*) in a manner virtually identical to OTF-1 (nucleotides −35 to −55) (*15*). The single nucleotide difference in the end points of the footprints is not significant; in both cases the discrepant nucleotide is in a region of the sequence that is resistant to DNase digestion, so the degree of protection is difficult to determine precisely.

To confirm that the purified preparation of OTF-1 contains a single protein species that binds to both the Ad Ori and the H2b promoter, we carried out competition experiments. In these experiments the gel electrophoresis–DNA binding assay was used to determine the relative affinity of OTF-1 for various DNA sequences (*16, 17*). The H2b DNA fragment could compete for the protein that bound to the marker Ad Ori fragment (Fig. 2B). Nearly equimolar quantities of Ad Ori fragment and H2b fragment are required to reduce the amount of bound marker fragment by 50%. DNA fragments containing base substitution mutations within the consensus octamer sequence in the Ad Ori or the H2b promoter had significantly lower affinity for this protein relative to either wild-type fragment. These data confirm that a single protein species in the preparation of pure OTF-1 had a high affinity for both the H2b promoter and the Ad Ori, and that this protein specifically recognized a DNA sequence within the octamer.

The ability of OTF-1 to activate initiation of adenovirus DNA replication was tested in

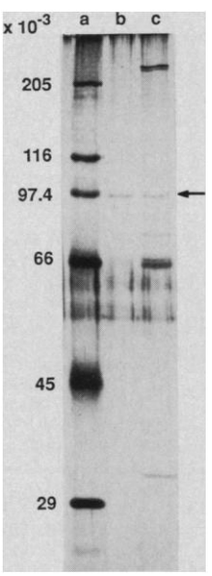

Fig. 1. SDS-PAGE of OTF-1 and NF-III. Samples of purified OTF-1 (*15*) and NF-III (*10*) each containing 43 U of Ad Ori DNA-specific binding activity (*10*) were analyzed by electrophoresis on an 8% SDS-polyacrylamide gel (*23*). After electrophoresis, the polypeptides were visualized by silver staining (*24*). The molecular size standards (relative molecular mass, M_r) were myosin (205,000), β-galactosidase (116,000), phosphorylase B (97,400), bovine serum albumin (BSA) (66,000), ovalbumin (45,000), and carbonic anhydrase (29,000). Lane a, molecular size standards; lane b, OTF-1; lane c, NF-III.

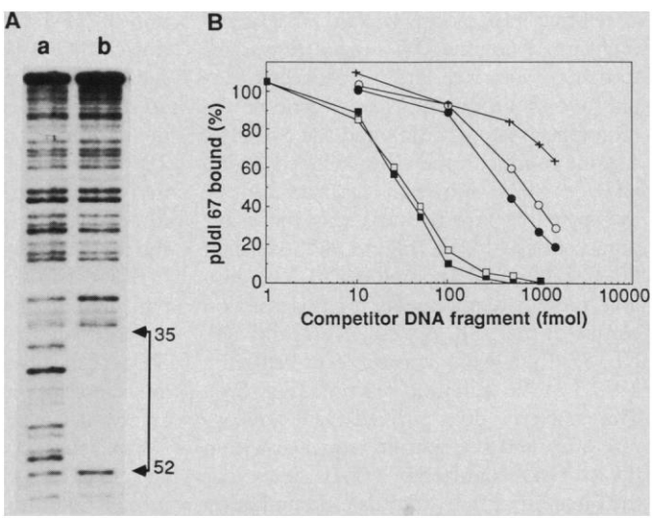

Fig. 2. (**A**) DNase I footprinting of OTF-1 bound to the Ad Ori. The plasmid pUdl 67 (*25*) was linearized with Bam HI, end-labeled with polynucleotide kinase, then restricted with Pvu II. The 157-bp fragment containing the Ad Ori was isolated by electrophoresis on an 8% polyacrylamide gel followed by electroelution. This fragment was manipulated as follows and then analyzed by electrophoresis as previously described (*10*). Lane a, DNA fragment subjected to DNase I (0.05 U of DNase, 2.5 fmol of DNA, 6-μl reaction volume); lane b, DNA fragment subjected to DNase I (as in lane a) in the presence of 22 Ad Ori–specific DNA binding units of OTF-1. The location of the protected sequence is identified by the bracket as bases 35 to 52 of the Ad Ori. (**B**) Competition of DNA fragments containing the Ad Ori or H2b gene promoter for binding to OTF-1. Relative affinities of potential octamer sequences for purified OTF-1 were determined by competition analysis by use of the gel electrophoresis DNA binding assay (*17, 26*). The radioactive marker fragment containing the entire Ad Ori (bp 1 to 67) was derived from pUdl 67 (*25*). The labeling and purification of this 157-bp fragment, as well as the assay conditions, can be found in (*10*). Competitor fragments were: □, the 157-bp Bam HI–Pvu II fragment derived from pUdl 67 (wild-type Ad Ori); ○, the 157-bp Bam HI–Pvu II fragment derived from pUpm 46 (mutant Ad Ori) (*27*); ■, 170-bp Hind III fragment derived from p16.3 (wild-type H2b promoter) (*15*); ●, 170-bp Hind III fragment derived from poMA (mutant H2b promoter) (*27*); +, the 90-bp Bam HI–Pvu II fragment derived from pUC 9 (*25*).

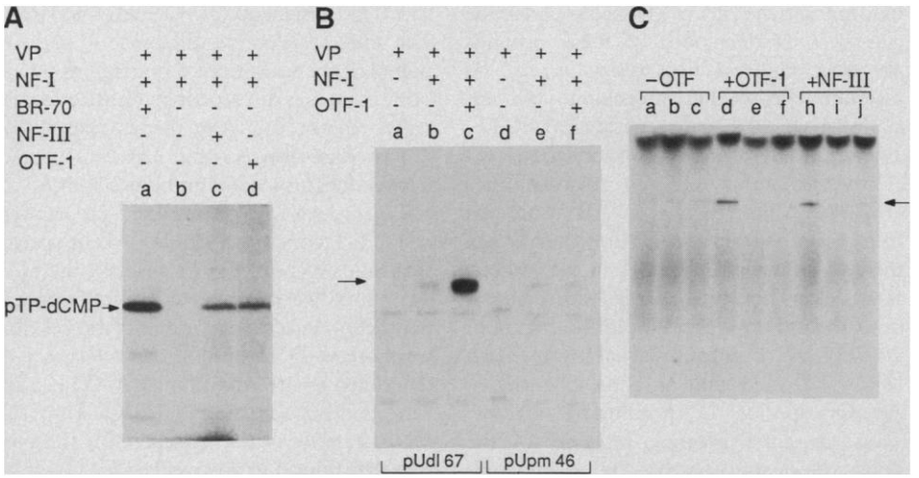

Fig. 3. (**A**) Initiation of adenovirus DNA replication is dependent on OTF-1 (*28*). Reaction mixtures contain adenovirus DNA-protein complex as template. VP, pTP and Ad Pol (1.2 μg), was purified as described (*9*); NF-1 (20 ng) was purified as described (*7*); BR-70, Bio-Rex 70 flow through fraction (3.1 μg) prepared as described (*9*); NF-III (3.4 Ad Ori binding units) was purified as described (*10*); and OTF-1 (1.7 Ad Ori binding units) was purified as described (*15*). The arrow indicates the location of the product of this reaction, a covalent complex between the 80-kD pTP and [α-^{32}P]deoxycytidine phosphate (dCMP) (*4*). (**B**) Stimulation of adenovirus DNA replication by OTF-1 is dependent on the presence of the NF-III binding site within the Ad Ori (*28*). The template DNAs pUdl 67 (*25*) (lanes a to c) or pUpm 46 (*27*) (lanes d to f) were incubated with combinations of purified protein components. Lanes a and d, viral proteins (VP) [pTP and Ad Pol (3.9 μg), 0.017 polymerase units, DBP 0.12 μg] purified as described (*9*); lanes b and e, VP with NF-I (25 ng); lanes c and f, VP with NF-I and OTF-1 (13.6 Ad Ori binding units). The arrow marks the position of the major replication product, which consists of a covalent complex between the largest DNA fragment and the pTP (*25*). (**C**) In vitro transcription from the H2b promoter depends on either OTF-1 or NF-III. Reconstituted transcription reactions were carried out as previously described (*15*). Equal specific DNA binding units of OTF-1 or NF-III were used as indicated. Template DNAs used contained H2b-derived promoters fused to the G minus cassette. Lanes a, d, and h, wild-type promoter (bp −178 to −19); lanes b, e, and i, H2b promoter deleted to bp −36. Detailed descriptions of these templates have been published (*15*).

a cell-free replication system. The initial event in adenovirus DNA replication is the formation of an ester linkage between the α-phosphoryl group of 2′-deoxycytidine 5′-monophosphate (dCMP) and the β-OH of a serine residue in the virus-encoded 80-kD pTP (*4*). The initiation reaction can be monitored in vitro by measuring the incorporation of [α-^{32}P]dCTP into SDS-resistant 80-kD pTP-dCMP complexes (*4*). The minimal protein requirements for this reaction consist of the pTP, Ad Pol, NF-I, and NF-III (*8, 10*). OTF-1 is capable of replacing NF-III in the initiation reaction (Fig. 3A). Thus not only does purified OTF-1 recognize and bind the specific sequence within the Ad Ori recognized by NF-III, it also has the same capacity to stimulate the initiation of adenovirus DNA replication.

It has been observed that the most efficient reconstituted adenovirus DNA replication reaction requires purified pTP, Ad Pol, DBP, NF-I, and NF-III (*8, 18*). We used a reconstituted in vitro replication reaction to show that the NF-III binding site within the Ad Ori is required for OTF-1 to stimulate viral DNA replication (Fig. 3B). When the replication template contains a wild-type Ad Ori, the reaction is dependent on the addition of NF-I and is stimulated by the addition of OTF-1 (Fig. 3B, lanes a to c). A base substitution within the Ad Ori at position 46 reduces the affinity of OTF-1/NF-III for the Ad Ori by approximately 20-fold (Fig. 2B). When a template containing this mutation is used in the reconstituted replication reaction, OTF-1 no longer has any effect on the reaction (Fig. 3B, lanes d to f).

Efficient in vitro transcription from wild-type human histone gene H2b promoter depends on the presence of OTF-1 (*15*). NF-III can substitute for OTF-1 in stimulating transcription from the H2b promoter in an octamer-dependent manner (Fig. 3C).

From these data we conclude that OTF-1 and NF-III are identical. We have previously demonstrated that the DNA replication activity of NF-I and the transcription activity of CTF might reside in a single polypeptide (*14*). In the case of NF-III/OTF-1 it is even more likely that a single polypeptide shares both replication and transcription activities.

The finding that one protein can function in both replication and transcription reactions might have significant biological implications. Numerous observations have been reported suggesting a functional relationship between DNA replication and transcription. Transcription of histone genes is very tightly associated with DNA replication (*19*). This observation is particularly relevant since the transcriptional activity of OTF-1 has been implicated in the S phase–specific induction of the human histone H2b gene (*20*). There are reports that the transcriptional activity of cellular DNA can affect the timing of its replication (*21*), and that replication of DNA can affect its transcriptional activity (*22*). Transcription regulatory elements (especially enhancers) have been shown to stimulate the DNA replication of several eukaryotic viruses (*4*). It must be noted that despite this accumulation of circumstantial evidence linking DNA replication with transcription, it is quite possible that adenovirus has simply subverted several cellular transcription factors for purposes of its own replication.

The mechanisms by which NF-I/CTF or NF-III/OTF-1 activate either transcription or DNA replication are not yet known. Such proteins might activate DNA by changing its structure upon binding. Alternatively they might activate adjacent proteins (polymerases or other binding factors) by their proximity. Finally, some might serve as nucleation sites for the formation of replication or transcription complexes. The adenovirus in vitro replication reaction is a relatively well-defined system that will serve as a useful model system for the exploration of these various possibilities.

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17. In the gel electrophoresis–DNA binding assay a defined DNA fragment is incubated with the DNA binding protein and then the reaction mix is analyzed by electrophoresis through agarose as previously reported (*10*). DNA fragments bound to protein are identified by their reduced mobility relative to DNA fragments that have not been exposed to protein. Constant amounts of purified OTF-1 and an end-labeled, Ad Ori–containing DNA fragment were mixed with increasing amounts of various unlabeled test DNA fragments. The quantity of test DNA required in the reaction to decrease the amount of radioactive marker DNA bound to protein (shifted species) by a defined fraction (50%) is taken to be indicative of the relative affinity of OTF-1 for the test DNA.
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26. Unlabeled competitor DNA fragments were purified by gel electrophoresis. Various quantities of competitor DNA fragment were mixed with 5×10^{-15} mol of labeled marker fragment. The DNA was then mixed with 1.1 Ad Ori–specific DNA binding units of OTF-1, and incubated for 30 min. After electrophoresis of the reaction mix, the gel was dried and autoradiographed. The bands corresponding to bound and unbound DNA were excised from the gel and quantitated by liquid scintillation counting. The amount of radioactive DNA bound by OTF-1 in the absence of competitor DNA was determined, and the fraction of this DNA bound in the presence of competitor DNA was calculated.
27. The pUpm 46 contains the Ad Ori with an A to T transversion at base 46 in the NF-III binding site (C. R. Burrow and T. J. Kelly, unpublished construction). The poMA contains a double base substitution within the octamer consensus sequence, generating the novel sequence ATTCAACT (*15*).
28. The standard in vitro initiation reaction was performed as described (*10*). All reaction mixtures were adjusted to constant concentrations of NaCl (20 m*M*), glycerol (10%), Hepes (*p*H 7.5, 30 m*M*), and other buffer constituents. Reconstitution of the replication of DNA containing the cloned Ad Ori was performed by previously described techniques (*26*), with the following modifications. Each reaction contained 60 ng of plasmid DNA restricted with Eco RI and Ava II. All reactions were adjusted to constant concentrations of NaCl (20 m*M*), glycerol (8%), Hepes (*p*H 7.5, 40 m*M*), and other buffer constituents. The reactions were carried out at 37°C for 60 min. The largest restriction fragment (approximately 1450 bp) contains the adenovirus terminus positioned at one end.
29. This study was supported by PHS grant CA16519 from the National Cancer Institute (T.J.K.), PHS grant CA42567 (R.G.R.), NIH grant ROIGM32544 (N.H.), the Pew Trust to Rockefeller University, and a Pew Scholars Award (N.H.). E.O'N. was a Monsanto Co. postdoctoral fellow. C.F. is the recipient of NIH training grant AI07233.

18 April 1988; accepted 11 July 1988

Mitogen-Induced Replication of Woodchuck Hepatitis Virus in Cultured Peripheral Blood Lymphocytes

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Peripheral blood lymphocytes (PBLs) isolated from woodchucks chronically infected with the woodchuck hepatitis virus (WHV) carry low levels of nonreplicating WHV DNA. When PBLs from chronic carrier woodchucks were activated in culture with the generalized mitogen lipopolysaccharide (LPS), WHV DNA replication was initiated in cells obtained from one of three animals examined. Intracellular WHV core particles, containing WHV DNA replication intermediates, RNA/DNA hybrid molecules, and an active endogenous DNA polymerase, appeared 3 days after the start of LPS stimulation. After 5 to 7 days of LPS stimulation, WHV DNA–containing particles, which displayed the properties of intact, mature virions, were released into the culture medium. These studies provide evidence for reactivation of a latent WHV infection of circulating lymphoid cells and indicate that the presence of nonreplicating hepadnaviral DNA in lymphoid cells represents a potentially active infection following cellular activation.

A NUMBER OF ACUTE AND CHRONIC viral infections involve a lymphatic phase with early replication at the site of entry and in regional lymph nodes followed by spread to nonlymphatic tissue, where the primary disease is manifested (*1*). In two well-studied chronic infections, measles virus in subacute sclerosing panencephalitis (SSPE) (*2*, *3*) and human immunodeficiency virus (HIV) in acquired immunodeficiency syndrome (AIDS) (*4*, *5*), recovery of virus in culture requires cellular activation. Thus, the lymphoid system may serve as a site of viral amplification and as a reservoir of latent virus.

Hepatitis B virus (HBV) causes acute and chronic liver disease and has been epidemiologically linked to primary hepatocellular carcinoma (HCC) (*6*). Recent studies on HBV and other members of Hepadnaviridae have demonstrated viral infection of lymhoid cells and other extrahepatic tissues, although the role of such infections in the pathogenesis of virus-induced disease is poorly understood (*7*, *8*). WHV and its natural host, the eastern woodchuck (*Marmota monax*), constitute the relevant animal model system for the study of HBV-induced disease, especially HCC and lymphatic infections (*7–10*).

Active replication of HBV, WHV, and duck hepatitis B virus (DHBV) DNA occurs in the spleen and in the liver (*8*, *11–13*). However, hepadnavirus genomes in PBLs are usually found in a nonreplicating state (*7*, *8*, *11*, *13*). Such observations could indicate that the viral genomes in these cells are no longer capable of sustaining a complete virus life cycle. Alternatively, these viral genomes could be replication competent, but the host cell environments may not be compatible with viral DNA replication.

To determine if the WHV genomes in PBLs were replication competent in an activated host cell environment, we stimulated PBLs from three chronically infected woodchucks, in culture, with LPS (Sigma), concanavalin A (Con A) (Difco), phytohemagglutinin (PHA) (Difco), or human recombinant interleukin-2 (IL-2) (Amgen). All four mitogens induced significant blast formation (as determined by microscopic examination) and variable levels of thymidine incorporation into cellular DNA (Table 1) in all the PBL preparations examined. The levels of incorporated exogenous thymidine were relatively low when compared to other cell systems (*14*), although these levels were significantly above the incorporation observed in unstimulated cells (Table 1). Maximal thymidine incorporation was observed during the first 24 hours of culture in the presence of LPS and at day 3 of culture for the other mitogens. Different PBL preparations varied considerably in their mitogen response; no consistent differences in the overall patterns of thymidine incorporation were observed between PBLs from an uninfected woodchuck (WC178) and PBLs from the chronically infected animals (WC139, WC192, WC195) (Table 1).

LPS maintained the viability of lymphoid cells in culture for several days (*15*). LPS treatment of PBLs from WC192 stimulated cell division, as evidenced by a twofold increase in total cell number over a 7-day period. Because the mitogenic activity of LPS is limited almost exclusively to B cells (*16*), a minor component of the total PBL population, the observed increase in total cell number is significant. The number of viable cells (as determined by the exclusion

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