ity seen in the complete record.

The huon pine chronology also reveals numerous past episodes of persistently above- and below-average summer temperatures lasting ten or more years. Yet the coldest and warmest periods inferred from our tree-ring record apparently occurred within about 50 years of each other in the 20th century. This pattern of unusual cold followed by unusual warmth could strongly skew the evaluation of recent temperature trends in Southern Hemisphere instrumental records, which are relatively infrequent before 1900 (*13*). As this study shows, long, high-resolution paleotemperature records from the Southern Hemisphere can help in properly evaluating the current warming trend.

In many respects the cold event of the early 1900s is as interesting as the post-1965 warming in Tasmania. It is present in both land and marine instrumental temperature records covering much of the Southern Hemisphere (*7–10, 13, 17–19*). The temperature decline was apparently preceded by a circumpolar expansion of Antarctic pack ice and the irruption of numerous icebergs into normally ice-free low latitudes (*20*). Other manifestations of this anomaly were a decrease in scalar wind speed in the Tasmania–New Zealand sector (*17*) and circumpolar changes in middle latitude (40° to 50°S) sea-level pressure associated with oscillations in zonal flow with a wave number of zero (*21*). Thus, there appears to have been a significant reorganization of the Southern Hemisphere ocean-atmosphere system in the early 1900s. A similar event of lesser magnitude may have also occurred around A.D. 1190 on the basis of qualitatively similar changes in the huon pine record. Understanding the mechanisms responsible for these and other past climatic fluctuations could contribute greatly toward explaining the recent warming in the Southern Hemisphere.

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Rel-Associated pp40: An Inhibitor of the Rel Family of Transcription Factors

NATHAN DAVIS,* SANKAR GHOSH,*† DANIEL L. SIMMONS, PAUL TEMPST, HSIOU-CHI LIOU, DAVID BALTIMORE, HENRY R. BOSE, JR.

The Rel-associated protein pp40 is functionally related to IκB, an inhibitor of the transcription factor NF-κB. Purified pp40 inhibits the DNA binding activity of the NF-κB protein complex (p50:p65 heterodimers), p50:c-Rel heteromers, and c-Rel homodimers. The sequence of the complementary DNA encoding pp40 revealed similarity to the gene encoding MAD-3, a protein with mammalian IκB-like activity. Protein sequencing of IκB purified from rabbit lung confirmed that *MAD-3* encodes a protein similar to IκB. The sequence similarity between MAD-3 and pp40 includes a casein kinase II and consensus tyrosine phosphorylation site, as well as five repeats of a sequence found in the human erythrocyte protein ankyrin. These results suggest that *rel*-related transcription factors, which are capable of cytosolic to nuclear translocation, may be held in the cytosol by interaction with related cytoplasmic anchor molecules.

THE PRODUCTS OF THE PROTO-ONcogene c-*rel* and the *rel* viral oncogene are members of a family of transcription factors that include the two subunits of transcription factor NF-κB (p50 and p65) and the *Dro-sophila* maternal morphogen *dorsal* (*1–4*). These proteins share sequence homology over 300 amino acids at their NH_2-terminus, a region that includes the DNA binding and dimerization domains. These proteins specifically bind to DNA sequences that are the same or slight variations of the 10-bp κB sequence in the immunoglobulin κ light chain enhancer. This κB binding sequence is present in a number of cellular and viral enhancers, and c-*rel* associates with these sites in the human immunodeficiency provirus (*5*).

Among the Rel-related proteins, NF-κB has been studied most extensively. NF-κB, a heterodimer of p50 and p65 subunits (*6*), is present ubiquitously in an inactive form in the cytosol, but is a constitutively active, nuclear protein in mature B cells (*7*). The DNA binding activity of NF-κB is activated and rapidly transported to the nucleus in cells exposed to mitogens or growth factors (*6–9*). The inactive cytosolic form is bound to IκB, which was initially characterized as a protein that specifically inhibits DNA binding by NF-κB (*8*). Purification of IκB from cell extracts reveals two forms, the major form of 35 to 37 kD (IκB-α) and a minor form of 40 to 45 kD (IκB-β) (*10, 11*). Treatment with the detergent deoxycholate (DOC) disrupts this NF-κB:IκB complex, releasing NF-κB, which then binds to DNA (*8*). Thus NF-κB may act as a second messenger, as the stimulatory signals initiated at the cell surface are transduced to the nucleus through the phosphorylation of IκB.

The products of c-*rel* and v-*rel* are found in the cytosol of avian lymphocytes associated with a distinct set of cellular proteins (pp40, p70, p115, and p124) (*12–16*). The molecular size of the 40-kD phosphoprotein (pp40) is close to that of IκB. In addition,

N. Davis and H. R. Bose, Jr., Department of Microbiology and Cell Research Institute, University of Texas at Austin, Austin, TX 78712.
S. Ghosh and H.-C. Liou, Whitehead Institute for Biomedical Research, Cambridge, MA 02142.
D. L. Simmons, Department of Chemistry, Brigham Young University, Provo, UT 84602.
P. Tempst, Memorial Sloan Kettering Cancer Center, New York, NY 10021.
D. Baltimore, Whitehead Institute for Biomedical Research, Cambridge, MA 02142, and Rockefeller University, New York, NY 10021.

*The first two authors contributed equally to this work.
†Present address: Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510.

the detergent sensitivity of the Rel:pp40 association is similar to that of NF-κB:IκB (*12, 13*); c-Rel–containing complexes in cytosolic extracts can be activated to bind DNA by treatment with detergents, suggesting that an IκB-like protein may regulate c-Rel (*5, 17*). Therefore, the ability of purified pp40 to function in a manner similar to IκB was evaluated.

The effect of pp40 on the DNA binding activity of NF-κB was determined with pp40 purified from v-*rel*–transformed chicken lymphoid cells (*14*). IκB was purified from rabbit lung (*11*) and was standardized with pp40 on a silver-stained SDS-polyacrylamide gel. Both purified pp40 and IκB specifically inhibited DNA binding by affinity-purified NF-κB at approximately equal concentrations (Fig. 1A). Excess pp40 does not inhibit DNA binding by two other transcription factors, the B cell–specific octamer binding protein Oct-2 (*18*) and the immunoglobulin enhancer binding protein E47 (*19, 20*). The DNA binding activity of an NF-κB complex inhibited by pp40 was restored with DOC at a concentration similar to that reported for IκB (*8*) (Fig. 1B). Therefore, pp40 and IκB are functionally analogous proteins.

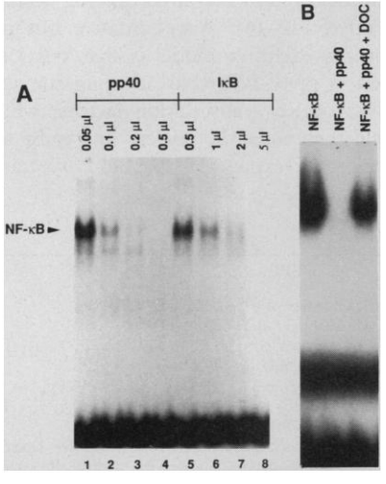

Fig. 1. Characterization of inhibitory activity of pp40. (**A**) Inhibition of purified NF-κB by pp40 and IκB. A fixed amount of NF-κB was titrated with increasing amounts of pp40 or IκB. Binding to a labeled DNA probe containing the κB site was assayed by electrophoretic mobility shift assay (*8*). Lanes 1 to 4, 0.05, 0.1, 0.2, and 0.5 µl of pp40; lanes 5 to 8, 0.5, 1, and 2 µl of purified IκB added to 1 µl of purified NF-κB. (The IκB protein concentration was approximately one-tenth that of pp40 as determined by silver staining.) (**B**) Reversal of pp40 inhibition of NF-κB by detergent. NF-κB (1 µl) was treated with 0.2 µl of purified pp40 in a nondissociating buffer that contained 0.2% DOC and 0.3% NP-40. The DOC concentration was then raised to 0.6% followed by the DNA binding mixture containing the labeled probe and sufficient NP-40 to give a final concentration of 1.5%. Lane 1, no pp40 or detergent; lane 2, pp40 but no detergent; lane 3, pp40 and detergent. The reaction mixtures were then analyzed by electrophoretic mobility shift assay (7).

To clone the cDNA that encodes pp40, the amino acid sequence of two tryptic peptides was obtained and used to synthesize oligonucleotide probes (*21*). Several positive clones were isolated from cDNA libraries made with mRNA prepared from chicken embryo fibroblasts (*22*). A 3.2-kb clone was further analyzed. To confirm that this clone encodes pp40, it was transcribed and translated in vitro. The translation product had an apparent molecular size of 40 kD and was immunoprecipitated by antiserum specific for pp40 (Fig. 2A). The ability of the protein to inhibit the DNA binding activity of NF-κB was also determined. Rabbit reticulocyte lysates used for in vitro translations contain an endogenous protein that binds to the κB site. This DNA-protein complex migrates at the position of authentic NF-κB; unlabeled wild-type but not mutated DNA probes compete with its formation (*20*). In addition, this complex can be inhibited in a detergent-sensitive manner by purified IκB and can be depleted with an antibody to the p50 subunit of NF-κB (*20*). On the basis of these criteria we consider this complex to contain rabbit NF-κB. Its presence in the lysates provided a convenient assay to determine IκB activity. Lysates programmed with the sense RNA of the pp40 clone inhibited the DNA binding activity of endogenous NF-κB (Fig. 2B). Therefore, immunological and functional analysis demonstrates that this clone encodes pp40.

We determined nucleotide and predicted amino acid sequences of the cDNA encoding pp40 (Fig. 3). An unusual feature of this clone is a long 5′ untranslated region (1539 bp). The open reading frame, which includes the sequences of the two pp40 tryptic peptides, begins at nucleotide 1540 and ends at nucleotide 2493. The 3′ untranslated region has one consensus polyadenylation signal (ATTAAA) located 17 bp upstream of the polyA tail and two repeats of the sequence ATTTA, which may signal rapid mRNA turnover (*23*).

The protein predicted from the open reading frame can be divided into three domains. The NH_2-terminal domain has a consensus phosphorylation site for casein kinase II (amino acids 35 to 40) and a consensus tyrosine phosphorylation site that is related to the binding domain for phosphatidylinositol-3 kinase (amino acids 43 to 51) (*24*). The middle portion of the protein consists of five repeats of a sequence found in human erythrocyte ankyrin as well as several tissue differentiation and cell cycle control proteins (*25*). These sequences mediate protein-protein interaction and may anchor proteins to the cytoskeleton. The COOH-terminal region contains sequences (PEST) that have been implicated in rapid protein turnover (*26*).

A cDNA clone encoding a protein that is induced rapidly upon monocyte adhesion has been identified (*27*). The size of its encoded product (35 to 37 kD), presence of ankyrin repeats, potential protein kinase C phosphorylation site, and ability to inhibit DNA binding by NF-κB has led to the suggestion that this gene, *MAD-3*, encodes an IκB-like activity. However, the absence of protein sequence from purified IκB or cross-reaction with specific antibodies prevented the unequivocal assignment as IκB. We obtained the amino acid sequence of two tryptic peptides derived from purified rabbit IκB-α (*28*). The first peptide has the sequence QQLTEDGDSFLHLAIIHEE, which is identical to pp40 at positions 72 to 90 and to MAD-3 at positions 68 to 86. The sequence from the second peptide, IQQQLGQLTLENLQ correspond to sequences in MAD-3 (amino acids 265 to 278) but is not present in pp40. Therefore, these protein sequences provide additional evidence that the MAD-3 protein is IκB.

Comparison of the amino acid sequences of pp40 and MAD-3 showed a similarity over a large portion of the proteins (Fig. 4).

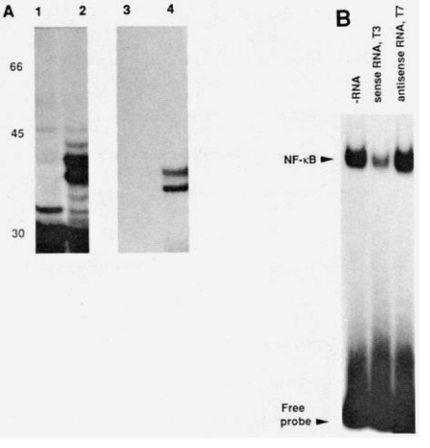

Fig. 2. Characterization of the pp40 cDNA. (**A**) The pp40 cDNA cloned in the Eco RI site of Bluescript was linearized with Hind III or Xba I. The antisense RNA was made with T7 RNA polymerase and the sense message transcribed with T3 RNA polymerase. The in vitro translations were performed in rabbit reticulocyte lysates, and the products were analyzed by SDS–polyacrylamide gel electrophoresis (*30*). Lane 1, translation of the antisense message; lane 2, translation of the sense message; lane 3, immunoprecipitation with antiserum to pp40 and the antisense translation products; lane 4, immunoprecipitation of the sense translation with antiserum to pp40. (**B**) Translation products of sense and antisense mRNAs of pp40 were used in an IκB assay with a labeled DNA probe containing the 10-bp κB site.

This similarity begins at the NH_2-terminal domain encompassing the consensus phosphorylation sites and extends beyond the ankyrin repeats into the PEST sequences. The striking sequence similarities between the two proteins suggest a functional relation; thus the portions of the proteins with related amino acid sequences should include a domain that inhibits NF-κB activity. The protein kinase C (PKC) phosphorylation site present in the COOH-terminal region of MAD-3 is absent in pp40, indicating that pp40 and MAD-3 are members of a family of IκB molecules. The absence of a PKC phosphorylation site in pp40 suggests that they may be regulated by different kinase pathways.

Because cells contain multiple Rel transcription factors, the ability of pp40 and IκB to inhibit the DNA binding of these factors was evaluated. cDNAs encoding p50 (*2*), p65 (*3*), and c-Rel (p85) (*29*) were transcribed and translated in vitro. Both p50 and c-Rel bound to a DNA probe that contained the κB site (Fig. 5, lanes 4 and 10). p65 did not bind to DNA by itself (lane 7), but could form a complex with p50 to yield the p50:p65 NF-κB complex (lane 13). c-Rel could also interact with p50 (lane 16), and the heterodimer formed a DNA-protein complex with a mobility distinct from that of c-Rel alone. When tested in an IκB assay (*8*), both IκB and pp40 inhibited the DNA binding of purified NF-κB (lanes 2 and 3), recombinant p50:p65 NF-κB (lanes 14 and 15), c-Rel (lanes 11 and 12), and p50:c-Rel (lanes 17 and 18) complexes, but not p50 alone (lanes 5 and 6). These results suggest that different combinations of Rel-related proteins can be regulated by association with IκB-like molecules.

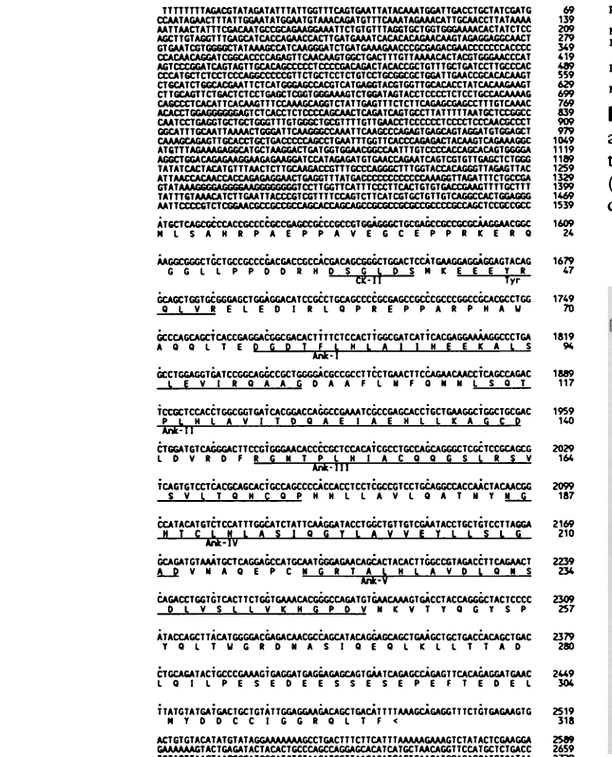

Fig. 3. Nucleotide and predicted protein sequence of pp40. The nucleotide sequence and amino acid sequence predicted from the open reading frame of the pp40-encoding cDNA is shown. The consensus tyrosine and casein kinase II phosphorylation sites are underlined. The five ankyrin repeats are indicated as Ank. I to V.

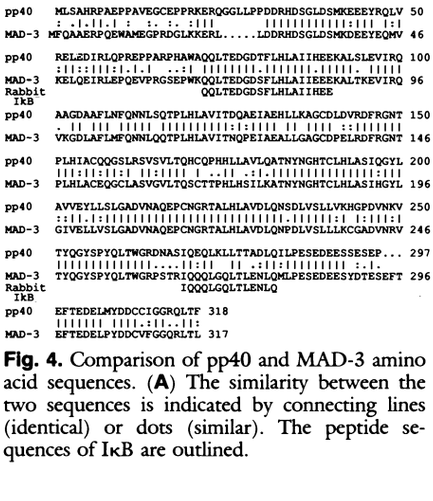

Fig. 4. Comparison of pp40 and MAD-3 amino acid sequences. (**A**) The similarity between the two sequences is indicated by connecting lines (identical) or dots (similar). The peptide sequences of IκB are outlined.

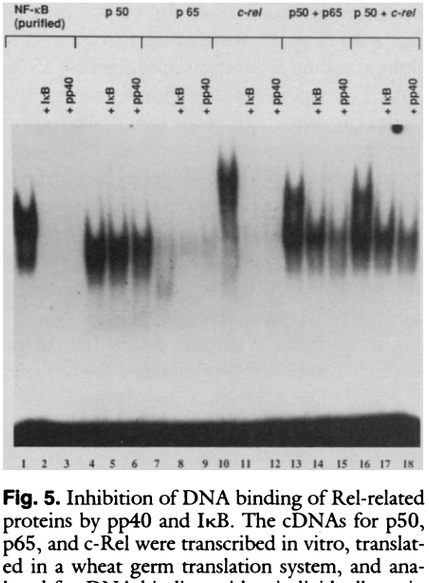

Fig. 5. Inhibition of DNA binding of Rel-related proteins by pp40 and IκB. The cDNAs for p50, p65, and c-Rel were transcribed in vitro, translated in a wheat germ translation system, and analyzed for DNA binding, either individually or in combinations, to a labeled DNA probe that contained the κB site (*30*). The effects of pp40 and IκB on DNA binding by these in vitro–translated proteins were tested under standard IκB assay conditions (*3*).

This report demonstrates that the pp40 protein, which associates with Rel-related proteins in chicken lymphoid cells is an IκB-like molecule. The finding that MAD-3and pp40 are related but not identical proteins indicates that a family of inhibitory proteins exists to regulate the family of Rel transcription factors. The existence of multiple inhibitory molecules provides the cell with greater control over the function of regulatory proteins that are expressed ubiquitously. The potential to assemble multiple Rel-family complexes that are controlled by a family of inhibitors might allow a graded response to exogenous stimulatory signals.

The finding that pp40 has IκB activity raises questions about the function of pp40 in the mechanism of transformation by v-*rel*. The v-*rel* oncogene product is a truncated version of the cellular homolog, c-*rel*, and lacks the domain essential for transactivation. The v-*rel* product inhibits transcription by binding to the promoter as an inactive complex (*5, 17*). Transformation by v-*rel* may, therefore, result from the inappropriate down-regulation of regulatory genes. Although v-Rel lacks the transactivation domain, it contains the pp40 binding domain of c-Rel (*12–16*). An alternative but not mutually exclusive model is that v-Rel sequesters pp40-IκB, thus allowing the activation of Rel transcription factors, which could then enter the nucleus and cause aberrant gene expression that leads to transformation.

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21. pp40 was purified from 50 liters of cultured REV-T– transformed lymphoid cells. Briefly, the cytosolic extract prepared by ultracentrifugation of the cell lysate was concentrated and subjected to gel exclusion chromatography on a Sephacryl S-200 column

(Pharmacia). The v-Rel–containing fractions were passed over an immunoaffinity column that contained an antibody to v-Rel (anti-pp59$^{v\text{-}rel}$). pp40 was eluted from the column in 1M $MgCl_2$. The purified protein was analyzed on SDS-PAGE, electrotransferred to nitrocellulose, and digested in situ with trypsin. The tryptic peptides were separated by high-performance liquid chromatography (HPLC), and two tryptic peptides yielded the following sequences: QAAGDAAFLNFQNNLSQTPLHLAVITDQAEIAEHLL and EPPARPHAWAQQLTEDGDTFLHLAIIHEE. The protein was sequenced by the Harvard Microchemistry Facility. The peptide sequences were used to generate partially degenerate guessmers. The resulting 44-nt probes had the following sequences: TTCCT(GC)AACTTCCAGAACAACCT(GC)(TA)G(TC)CAGACCCCCCT(GC)CACCT and TGGGC(GC)CAGCAGCAGCTGAC(CA)GAGGA(TC)GGCGA(CT)ACCTT(TC)CTGCACCT. These oligonucleotides were end-labeled with T4 polynucleotide kinase and used to screen cDNA libraries.

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28. Proteins electroblotted onto nitrocellulose were processed by tryptic digestion, and the resulting peptides were separated with narrow bore reversed-phase HPLC. Two of the peptide peaks were successfully sequenced with initial yields of 7 and 3 pmol.

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30. The templates for in vitro transcription–translation were obtained by linearizing the plasmids with the appropriate restriction enzymes. The linearized templates were then transcribed with T3, T7, or SP6 RNA polymerases. The purified RNAs were translated in a wheat germ extract with [^{35}S]methionine to obtain labeled proteins that were quantitated by SDS-PAGE and fluorography. The quantitation was done to ensure that equivalent amounts of proteins were used in the assays. The electrophoretic mobility shift assay was performed in 0.25× tris-borate-EDTA (1× TBE: 0.089 M tris, 0.089 M boric acid, 0.02 mM EDTA) to allow greater resolution of the homo- and heteromeric complexes.

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Cell Cycle Regulation of Histone H1 Kinase Activity Associated with the Adenoviral Protein E1A

ANTONIO GIORDANO, JOO HUN LEE, JUDITH A. SCHEPPLER, CHRISTINE HERRMANN,* ED HARLOW,† ULRICH DEUSCHLE, DAVID BEACH, B. ROBERT FRANZA, JR.‡

Several cellular proteins form stable complexes with the proteins encoded by the adenovirus early region 1A (E1A) gene in extracts derived from adenovirus infected or transformed cells. Two of the cellular proteins that bind to E1A have been identified; one, a 105-kilodalton protein (pRb), is the product of the retinoblastoma gene, and the other, a 60-kilodalton protein, is a human cyclin A. Two other proteins that bind E1A have now been shown to be related to p34^{cdc2}. This E1A complex displayed histone H1–specific kinase activity; the kinase activity was modulated during the cell division cycle, and association of pRb with E1A apparently was not required for this activity.

THE *CDC2* GENE ENCODES A 34-kD protein kinase (p34^{cdc2}) (*1*). In the fission yeast, *Schizosaccharomyces pombe*, *cdc2* is required in the G_1 stage of the cell cycle before DNA replication has begun and in G_2 at the beginning of mitosis (*2*). A *cdc2* homolog encodes a component of the maturation-promoting factor (MPF) of *Xenopus* eggs (*3*). The mammalian *cdc2* product is a protein kinase and is required for mitosis (*4*) and for the transition from G_1 to S phase (DNA replication) (*5*). The p34^{cdc2} protein activates the replication function of the SV40 T antigen (*6*). At least two complexes of mammalian p34^{cdc2} have been identified, the p62 (cyclin B)-p34^{cdc2} complex and the p60 (cyclin A)-p34^{cdc2} complex. The amount of p62-p34^{cdc2} complex correlates with a cell cycle–dependent histone H1 kinase activity that is most active in metaphase. The p60-p34^{cdc2} complex acts as a histone H1 kinase in interphase and metaphase (*7, 8*). Human p34^{cdc2} can form a complex with a 13-kD protein that probably is the product of a homolog of the fission yeast *suc1*$^+$ gene, a gene that rescues some but not all temperature-sensitive mutant alleles of *cdc2* in fission yeast (*9*), and is essential for cell cycle progression (*10*). The p13^{suc1} protein is a subunit of the p34^{cdc2} protein kinase in mammalian cells and in the budding yeast, *Saccharomyces cerevisiae* (*11*).

The E1A proteins are the first viral gene products synthesized after cells are infected with adenovirus. The E1A polypeptides function in both cellular transformation and regulation of gene expression (*12, 13*). For example, co-transfection of the E1A gene with another adenovirus early region gene product, E1B, or with an activated Ha-*ras* oncogene results in morphogenic transformation of either primary cells or proliferating tissue culture cells (*13*). Other activities of E1A include the induction of G_0-arrested mammalian cells to progress to DNA synthesis (*14*), the ability to induce tumor necrosis factor (TNF) sensitivity (*15*), and the prevention of transforming growth factor (TGF) β–mediated inhibition of cell growth (*16*).

Three regions, designated domains 1, 2, and 3, are highly conserved among E1A proteins from different adenovirus serotypes (*17*). Two sets of nuclear phosphoproteins arise from differential splicing of the E1A transcripts. The resulting proteins consist of 289 or 243 amino acids and are both post-translationally modified. The larger protein contains an internal sequence of 46 amino acids that has been identified as conserved domain 3 and appears to be sufficient for E1A-mediated transactivation of transcription (*18*).

The E1A conserved domain 2 and the NH_2-terminal region, including conserved domain 1 both contribute to transforming activity (*17, 18, 19*). Sites within these domains of E1A are required for complex formation with several specific cellular proteins (*20*). Two of these proteins have been identified; one of 105 kD (pRb) is the product of the retinoblastoma gene (*21*); and one of 60 kD (p60) is associated with only certain isoforms of p34^{cdc2} and is encoded by a cyclin A gene (*8, 22*). We now show that two cellular proteins in the E1A immune complex are either products of the human *cdc2* gene or a related gene, and that E1A immune complexes containing these proteins have a histone H1 kinase activity.

The cdc2-associated polypeptide, p60, is the same p60 protein that interacts with the adenovirus E1A protein (*8*). Four specific isoforms of p34^{cdc2} were present in immune complexes (*8*), isolated with a monoclonal antibody to p60 (C160). Two of these isoforms were recognized by an antiserum to the COOH-terminal portion of human p34^{cdc2} (G6). All four isoforms were recognized by an antibody to *S. pombe* p34^{cdc2} (G8) (*23*) and by p13-Sepharose. Proteins

A. Giordano, J. H. Lee, J. A. Scheppler, B. R. Franza, Jr., Freeman Laboratory of Cancer Cell Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.
C. Herrmann and E. Harlow, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.
U. Deuschle and D. Beach, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

*Present address: Division of Molecular Biology, Baylor College of Medicine, Houston, TX 77030.
†Present address: Department of Molecular Oncology, Massachusetts General East Cancer Center, Charlestown, MA 02129.
‡To whom correspondence should be addressed.